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(54) Title: PRE-HARVEST SPROUTING

(57) Abstract

The invention relates to materials and methods which may be used in the detection and manipulation of Pre-Harvest Sprouting (PHS) and other dormancy-related phenotypes in plants. Specifically disclosed are oat and wheat VP1 homologues (afVP1 and taVP1 respectively) plus also variants, particularly alleles of these. The sequence and mapping data provided can be used in plant breeding and/or in molecular-biology based methods to improve e.g. wheat varieties. Also disclosed are primers which are specific for orthologues, alleles or wheat-genomes plus methods of using these. Vectors, cells and transgenic plants are also provided, as are related products and methods of use.

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PRE-HARVEST SPROUTING

TECHNICAL FIELD

The present invention relates to materials and methods which may be used in the detection and manipulation of Pre-Harvest Sprouting (PHS) and other dormancy-related phenotypes in plants. The present invention also relates to materials and methods for use in plant breeding - in particular to molecular-biology based methods for generating, identifying, characterising or manipulating genetic variation which affects the PHS and other dormancy related traits.

PRIOR ART

Pre-Harvest Sprouting (PHS)

Pre-Harvest Sprouting (PHS) of non-dormant grains is a major limiting factor in achieving consistent bread making quality of UK wheat. Average annual losses due to PHS in the UK wheat crop have been estimated at some £17 million, but the problem is erratic and is much more severe in cool, damp seasons. Variation in the degree of sprouting damage from year to year makes this problem difficult to select against in conventional breeding programmes.

VP1 in maize and other species

Previous work in other plant species has shown that the VIVIPAROUS-1 gene is a major regulator of embryo maturation in maize. Thus McCarty et al (1989) in The Plant Cell 1, 523-532 disclosed that *vp1* mutants in maize were abscisic acid (ABA) insensitive, and demonstrated its role in controlling the developmental responses associated with the maturation phase of seed formation.

VP1 mutants were shown to germinate precociously. Similarly McCarty et al (1991) in Cell 55, 895-905 disclosed that *VP1* encoded 73 kDa transcription factor.

Giraudat et al (1992) in The Plant Cell 4, 1251-1261 showed that Arabidopsis ABI3 mutants had altered seed development & germination. The predicted gene product was similar to VP1 protein. These results and others show that *ABI* 3/*VP1* function as developmental regulators during the maturation stage of embryogenesis by regulating transcription of sets of genes that determine the embryonic phenotype in preparation for desiccation of the seed prior to shedding.

A VP1 homolog in rice has also been isolated (Hattori et al (1994) Plant Molecular Biology 24, 805-810). Similarly in Abstract, Poster No P184 "Poaceae sequence analysis: cloning of a VP-1 homolog from genomic barley DNA via PCR", at the Plant and Animal Genome V Conference in San Diego, USA, January 1997, Wilson & Sorrells disclosed the use of conserved primers to pick out vp1 homologs in Barley.

Wilson speculated that a wheat VP1 homolog may be obtainable by comparison with maize, rice and barley sequences, and (on the basis of comparison with these species) that the R locus may contain a wheat VP1 homolog.

Interestingly, earlier work by Cadle et al (1994) in Genome 37, 129-132 had already shown that the maize *vp1* from McCarty didn't hybridise strongly to wheat DNA and could not therefore be used as a probe to map the wheat gene, although various ABA-inducible genes were mapped successfully.

Dormancy

Other recent studies of the genetics of the transition from embryogenesis to germination in maize and Arabidopsis show that mutation of GA (gibberellic acid) and ABA synthesis and sensitivity can alter dormancy levels (Koornneef and Karssen, 1994). For example, whereas the Arabidopsis mutation ga 1 causes a loss of germination due to GA deficiency, aba/abi mutations (that affect ABA synthesis and perception respectively) cause a loss of dormancy (and in strong alleles, loss of viability) because embryos fail to develop desiccation tolerance during maturation (for example alleles of abi 3, Ooms et al., 1993).

It has been suggested that PHS in wheat is the result of the lack of induction of dormancy during embryo development (Gale and Lenton 1987). As is known to those skilled in the art, dormancy is one of two possible developmental states which mature seeds may show following desiccation and shedding (the other being germination). Embryo dormancy develops during late embryogenesis, and results in a lack of germination. Following imbibition of the mature shed seed it results in an inactive phase of plant growth during which development is deferred, although the embryo still maintains a high metabolic activity. Dormancy of mature imbibed seeds occurs even under environmental conditions that would favour germination, indicating that the process is not simply a lack of correct conditions. During dormancy, cells within the mature embryo are maintained in an arrested state, and nuclear DNA values obtained from A. fatua embryos indicate that the cell-cycle is held in G1 and DNA replication does not occur during imbibed dormancy (Elder and Osborne 1993). Dormancy is probably an evolutionary strategy that allows survival of seeds through adverse conditions, and

dispersal of seed germination through time. Dormancy is therefore a very important phase of plant development required both for the inhibition of germination prior to completion of embryogenesis, and for the pre-germinative survival of mature seeds. It is also an important agronomic trait, with the market value of wheat being determined, *inter alia*, by its Hagberg Falling Number, which measures the degree to which some germination-related processes have progressed (discussed in relation to plant breeding hereinafter).

Seeds of the persistent weed A. fatua can show very high levels of embryo dormancy (Simpson 1978).

Embryos with primary dormancy go through a time and environment sensitive process of after-ripening in the dry seed, that is manifested by loss of dormancy in the imbibed seed (Mayer and Poljakoff-Mayber 1989). Dormancy can subsequently be reimposed on after-ripened dry embryos under specific environmental conditions ('induced' or 'secondary' dormancy). These features indicate that signals perceived by the dry seed influence developmental choices following imbibition, resulting in either dormancy or germination (Hilhorst and Karssen 1992). Recent work on the water status of embryos of A. fatua has demonstrated that individual enzymatic and non-enzymatic reactions, rather than metabolic processes control this dormancy/non-dormancy switch in the dry seed (Foley 1994). Others have proposed that the process may involve kinase-phosphatase interactions (Trewavas 1987).

Many studies have analysed the genetic control of embryo dormancy (Hilhorst and Karssen 1992). Results obtained from experiments with inbred lines of A. fatua have suggested that in this species dormancy may be controlled by three loci, two that promote dormancy (L1 and L2), and one that promotes after-ripening (E) (Jana et al., 1979,

Jana et al. 1988). These dormancy genes have not been cloned or characterised, their existence was inferred from statistical analysis of segregation for dormancy phenotypes among the progeny from a cross between two different strains.

There is currently a requirement for materials and methods which have utility in the identification or molecular tagging of the genes responsible for PHS in wheat, or which could be used in the manipulation of the PHS trait in wheat or other plants.

Thus it can be seen that the provision of such materials or methods would provide a contribution to the art.

DISCLOSURE OF THE INVENTION

The present inventors have for the first time identified a gene from the oat *Avena fatua* which encodes a protein which has a high degree of similarity to known VP1/ABI 3 related transcription factors. The expression product of this gene has been termed afVP1.

By studying imbibed mature seeds, the expression of afVP1 has been correlated with the dormant phenotype (primary dormancy, secondary dormancy and after-ripening) in oat. In particular, the present inventors have demonstrated that wild oat has the potential for extremely high levels of dormancy in the mature dry seed, and that expression of afVP 1 is absolutely correlated to the dormant phenotype in imbibed mature seeds. This is the first demonstration of expression of a VP 1-homologue in a developmental situation other than embryogenesis. It indicates that afVP1 activity keeps mature seeds dormant, and inhibits germination - it can thus be used to maintain or impose sufficient intensity and duration of dormancy to avoid PHS before harvest.

In addition to establishing an important role for afVP1 in the control of after-ripening and both primary and secondary dormancy. The present inventors have also employed afVP1 to identify the hitherto unobtained wheat VP1 homolog (hereinafter taVP1) and map its genomic position. As will be discussed in more detail below, the information made available by the present invention has important and industrially applicable implications for the detection and manipulation of PHS and other dormancy related traits in plants, and especially PHS in wheat. In particular work done by the inventors indicates that the ability to keep mature seeds dormant, and inhibit germination, has been lost by the wheat VP 1 due to breeding since domestication which has favoured the evolution of a crippled wheat VP 1 that cannot impose high levels of dormancy (resistance to PHS) on the mature seed.

Introduction of the wild oat afVP 1 into wheat can therefore be used to induce higher levels of dormancy (and thus resistance to PHS) in wheat as afVP 1 compensates for the crippled function of wheat VP 1.

Thus in a first aspect of the present invention there is provided a nucleic acid molecule, encoding afVP1, and having the sequence set out in Seq ID No 1 (shown in Fig 4(a)). A further afVP1 sequence, differing slightly from Seq ID No 1, has been deposited in the EMBL database under accession number AFJ001140 after the priority date of the present application.

The existence of an oat homologue to maize VP1 was reported briefly in a poster by M.J.Holdsworth "Dormancy-related expression of the wild oat (*Avena fatua*) homolog of the maize gene *Viviparous 1 (Vp1)*". Abstract, Poster No.49, Seventh International Symposium on Pre-Harvest Sprouting in Cereals 1995, Abashiri, Japan, July 1995.

Some comments about its properties were made - however the precise means of cloning the homolog, its sequence, and specific applications for it, were not disclosed.

Additionally Jones et al in April 1997 disclosed that afVP1 was correlated with primary and secondary dormancy (see Jones et al (1997) J Exp Bot 48 (Suppl) 45). Once again no details about how afVP1 could be obtained, or particular applications for it, were disclosed.

The nucleic acid molecules and their encoded polypeptide products (see below) according to the present invention may be provided isolated and/or purified from their natural environment, in substantially pure or homogeneous form, or free or substantially free of nucleic acid or genes of the species of interest or origin other than the sequence encoding a polypeptide with the required function. Nucleic acid according to the present invention may include cDNA, RNA, genomic DNA and may be wholly or partially synthetic.

The term "isolate" encompasses all these possibilities. Where a DNA sequence is specified, e.g. with reference to a figure, unless context requires otherwise the RNA equivalent, with U substituted for T where it occurs, is encompassed.

By virtue of its demonstrated properties, the nucleic acid of the first aspect may has utility, inter alia, in producing transformed crop plants having desirable primary or secondary dormancy, or after-ripening, properties, and in particular may be resistant to PHS.

In a further (second) aspect of the invention there is disclosed variants of the sequence provided, which may for instance be mutants or other derivatives, or naturally occurring alleles (or other homologues,

including orthologues) of the sequence.

In the case of mutants and derivatives, the variant encodes a product which is homologous to the sequence of Seq ID No 1, and preferably which retains a functional characteristic that the product encoded by the variant has the afVP1 activity.

By 'afVP1 activity' is meant the ability to act as a transcription factor which is capable of activating some or preferably all of the genes which are activated by afVP1 (e.g. Em, C1) and repressing genes which are repressed by afVP1 (e.g. alpha-amylase - see Hoeker et al 1995 for maize VP1 activity in this regard). This can be assayed either directly using e.g. a reporter gene system linked to any of these genes or their promoters. Alternatively it may be assayed by preparing transformed plants and assaying its phenotypic effects *in vivo* (i.e. alteration of dormancy as described above).

Methodology for such transformation is described in more detail hereinafter.

Methods for producing such mutants or derivatives based on the sequence provided, and for identifying alleles (or other homologs) and then assessing homology are discussed below, and form one part of this aspect of the invention.

In all cases the nucleic acid molecule which is the mutant or other derivative is ultimately generated either directly or indirectly (e.g. via one or more amplification or replication steps) from oat afVP1 (including alleles thereof), preferably from a nucleic acid molecule comprising all or part of sequence ID No 1.

Changes to a sequence, to produce a mutant or derivative, may be by one or more of addition, insertion, deletion or

substitution of one or more nucleotides in the nucleic acid, leading to the addition, insertion, deletion or substitution of one or more amino acids in the encoded polypeptide. Specifically included are parts or fragments (however produced) corresponding to portions of the sequences provided, and which encode polypeptides having afVP1 activity.

Changes may be desirable for a number of reasons, including introducing or removing the following features: restriction endonuclease sequences; other sites which are required for post translation modification; cleavage sites in the encoded polypeptide; motifs in the encoded polypeptide for post translational modification. All of these may assist in efficiently cloning and expressing an active polypeptide in recombinant form (as described below).

Other desirable mutation may be random or site directed mutagenesis in order to alter the activity (e.g. specificity) or stability of the encoded polypeptide. Particular regions of interest may be those which correspond to the regions of VP 1 which have been shown to function as either a transcriptional activation domain (amino acids 28-121, McCarty *et al.* 1991), or as a repressor domain (amino acids 238-375, Hoecker *et al.* 1995). Sections of these regions are highly conserved amongst all the VP 1 homologues (see Figure 4, and discussion in the Examples) indicating that these sections may provide function to these regions. Comparison of the BR2 region, (shown previously to interact with other classes of transcription factors [Hill *et al.* 1996]), shows a high degree of similarity between afVP 1 and other homologues, indicating a conservation of function for this part of the protein. These comparisons (in terms of predicted amino acid structure) are shown in Figure 4.

Those regions which differ from the corresponding parts of other *VP1*s may also be of interest in that they may be responsible for the high dormancy demonstrated for oat.

Specifically embraced are derivatives which are truncated, or which have functional regions replaced with corresponding regions from other sources.

As is well-understood, homology at the amino acid level is generally in terms of amino acid similarity or identity.

Similarity allows for conservative variation, i.e. substitution of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. As is well known to those skilled in the art, altering the primary structure of a polypeptide (in this case the afVP1 protein) by a conservative substitution may not significantly alter the activity of that peptide because the side-chain of the amino acid which is inserted into the sequence may be able to form similar bonds and contacts as the side chain of the amino acid which has been substituted out. This is so even when the substitution is in a region which is critical in determining the peptides conformation.

Also included are homologs generated from afVP1 having non-conservative substitutions. As is well known to those skilled in the art, substitutions to regions of a peptide which are not critical in determining its conformation may not greatly affect its activity because they do not greatly alter the peptide's three dimensional structure. In regions which are critical in determining the peptides conformation or activity such changes may confer advantageous properties on the polypeptide. Indeed,

changes such as those described above may confer slightly advantageous properties on the peptide (e.g. altered stability, or DNA binding efficiency).

Similarity or homology (or identity, the terms are used synonymously) may be as defined and determined by the TBLASTN program, of Altschul *et al.* (1990) *J. Mol. Biol.* 215: 403-10, which is in standard use in the art, or, and this may be preferred, the standard program BestFit, which is part of the Wisconsin Package, Version 8, September 1994, (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA, Wisconsin 53711). BestFit makes an optimal alignment of the best segment of similarity between two sequences. Optimal alignments are found by inserting gaps to maximize the number of matches using the local homology algorithm of Smith and Waterman.

Thus a mutant, derivative or allele (or other homolog) of the present invention shares homology with afVP1. Homology may be at the nucleotide sequence and/or amino acid sequence level. Preferably, the nucleic acid and/or encoded amino acid sequence shares homology with the coding sequence or the sequence encoded by the nucleotide sequence of Seq ID No 1, preferably at least about 50%, or 60%, or 70%, or 80% homology, most preferably at least about 90%, 95%, 96%, 97%, 98% or 99% homology.

Homology may be over the full-length of the relevant sequence shown herein, or may more preferably be over a contiguous sequence of about or greater than about 20, 25, 30, 33, 40, 50, 67, 133, 167, 200, 233, 267, 300, 333, 400, 450, 500, 550, 600 or more amino acids or codons, compared with the relevant amino acid sequence or nucleotide sequence as the case may be.

Thus a variant amino acid sequence in accordance with the present invention may include within the sequence shown

in Figure 4, a single amino acid change with respect to the sequence shown in Figure 4, or 2, 3, 4, 5, 6, 7, 8, or 9 changes, about 10, 15, 20, 30, 40 or 50 changes, or greater than about 50, 60, 70, 80 or 90 changes. In addition to one or more changes within the afVP1 amino acid sequence shown in Figure 4, a variant amino acid sequence may include additional amino acids at the C-terminus and/or N-terminus. Naturally, changes to the nucleic acid which make no difference to the encoded amino acid sequence (i.e. 'degeneratively equivalent') are included.

A further part of the present invention provides a method of identifying and cloning further afVP1 homologues or alleles from plant species which method employs a nucleotide sequence as described above.

As mentioned earlier, the present inventors have already used this method to identify a hitherto unidentified wheat *VP1* homolog (termed taVP1) - this notwithstanding the failure of earlier workers (e.g. Cadle et al (1994)) who used maize *VP1* to probe wheat. Nucleic acid molecules identified using this method form one part of the second aspect of the invention e.g. taVP1, as do mutants and derivatives of those genes.

The originally derived nucleotide sequences of various clones of taVP1 are shown in Figures 6 and 8.

Later sequences for the fully sequenced clone (designated taVP1 - referred to as Seq ID No 2) and clones 2, 3, 4, 5, 6, and 9 are shown in Fig10(a)-(g).

It is apparent the sequences shown that none of them encodes a full length protein i.e. they appear to be crippled to various degrees. This has important implications for improving dormancy in wheat, as regards

molecular biology-based methods for transforming or breeding improved plants.

It may be noted that McKibbin et al, in a poster at SEB meeting in April 1997 reported using the wild oat homolog of VP1 in N-blots of wheat mRNA from various cultivars to demonstrate that expression levels of certain mRNAs were reduced in non-dormant phenotypes (see McKibbin et al (1997) J Exp Bot 48: (Suppl) 47). However no data regarding the sequence of the putative wheat (or oat) homolog(s) was presented.

When identifying homologs using the strategies below, if need be clones or fragments identified in the search can be extended to produce full length molecules. For instance if it is suspected that they are incomplete, the original DNA source (e.g. a clone library, poly(A)RNA extracted from embryos) can be revisited to isolate missing portions e.g. using sequences, probes or primers based on that portion which has already been obtained to identify other clones containing overlapping sequence.

In one embodiment, nucleotide sequence information provided herein may be used in a data-base (e.g. of ESTs) search to find homologous sequences, expression products of which can be tested for ability as described below.

In a further embodiment, a homolog or allele in accordance with the present invention is also obtainable by means of a method which includes:
(a) providing a preparation of nucleic acid, e.g. a genomic or cDNA library),
(b) providing a nucleic acid molecule having a nucleotide sequence shown in or complementary to a nucleotide sequence shown in Seq ID No 1 preferably from within the coding sequence (i.e. encoding for the afVP1 amino acid sequence shown in Fig 4), most preferably the probe used

is distinctive or characteristic of afVP1 rather than other, known, VP1 analogs,

(c) contacting nucleic acid in said preparation with said nucleic acid molecule under conditions for hybridisation of said nucleic acid molecule to any said gene or homologue in said preparation, and identifying said gene or homologue if present by its hybridisation with said nucleic acid molecule.

"Distinctive" or "characteristic" regions of afVP1 can be determined with reference to comparisons with know VP 1 homologues, for instance those shown in Fig 4. Such regions and specific oligonucleotides are found away from the conserved regions which are described in more detail in Example 2 below, and will allow those skilled on the art to design probes and primers which will not hybridise with prior art sequences.

Probing may employ the standard Southern blotting technique. For instance DNA may be extracted from cells and digested with different restriction enzymes. Restriction fragments may then be separated by electrophoresis on an agarose gel, before denaturation and transfer to a nitrocellulose filter. Labelled probe may be hybridised to the DNA fragments on the filter and binding determined. DNA for probing may be prepared from RNA preparations from cells.

Test nucleic acid may be provided from a cell as genomic DNA, cDNA or RNA, or a mixture of any of these, preferably as a library in a suitable vector. If genomic DNA is used the probe may be used to identify untranscribed regions of the gene (e.g. promoters etc.), such as is described hereinafter.

Preliminary experiments may be performed by hybridising under low stringency conditions. For probing, preferred

conditions are those which are stringent enough for there to be a simple pattern with a small number of hybridisations identified as positive which can be investigated further.

It is well known in the art to increase stringency of hybridisation gradually until only a few positive clones remain. Suitable conditions would be achieved when a large number of hybridising fragments were obtained while the background hybridisation was low. Using these conditions nucleic acid libraries, e.g. cDNA libraries representative of expressed sequences, may be searched. Those skilled in the art are well able to employ suitable conditions of the desired stringency for selective hybridisation, taking into account factors such as oligonucleotide length and base composition, temperature and so on.

For instance, screening may initially be carried out under conditions, which comprise a temperature of about 37°C or less, a formamide concentration of less than about 50%, and a moderate to low salt (e.g. Standard Saline Citrate ('SSC') = 0.15 M sodium chloride; 0.15 M sodium citrate; pH 7) concentration.

Alternatively, a temperature of about 50°C or less and a high salt (e.g. 'SSPE'= 0.180 mM sodium chloride; 9 mM disodium hydrogen phosphate; 9 mM sodium dihydrogen phosphate; 1 mM sodium EDTA; pH 7.4). Preferably the screening is carried out at about 37°C, a formamide concentration of about 20%, and a salt concentration of about 5 X SSC, or a temperature of about 50°C and a salt concentration of about 2 X SSPE. These conditions will allow the identification of sequences which have a substantial degree of homology (similarity, identity) with the probe sequence, without requiring the perfect homology for the identification of a stable hybrid e.g.

70% homology.

Suitable conditions include, e.g. for detection of sequences that are about 80-90% identical, hybridization overnight at 42°C in 0.25M Na_2HPO_4, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 55°C in 0.1X SSC, 0.1% SDS. For detection of sequences that are greater than about 90% identical, suitable conditions include hybridization overnight at 65°C in 0.25M Na_2HPO_4, pH 7.2, 6.5% SDS, 10% dextran sulfate and a final wash at 60°C in 0.1X SSC, 0.1% SDS.

Binding of a probe to target nucleic acid (e.g. DNA) may be measured using any of a variety of techniques at the disposal of those skilled in the art. For instance, probes may be radioactively, fluorescently or enzymatically labelled. Other methods not employing labelling of probe include amplification using PCR (see below), RN'ase cleavage and allele specific oligonucleotide probing. The identification of successful hybridisation is followed by isolation of the nucleic acid which has hybridised, which may involve one or more steps of PCR or amplification of a vector in a suitable host.

In a further embodiment, hybridisation of nucleic acid molecule to an allele or homologue may be determined or identified indirectly, e.g. using a nucleic acid amplification reaction, particularly the polymerase chain reaction (PCR). PCR requires the use of two primers to specifically amplify target nucleic acid, so preferably two nucleic acid molecules with sequences characteristic of afVP1 are employed. However, if RACE is used (see below) only one such primer may be needed.

PCR techniques for the amplification of nucleic acid are described in US Patent No. 4,683,195 and Saiki *et al.*

Science 239: 487-491 (1988). References for the general use of PCR techniques include Mullis et al, Cold Spring Harbor Symp. Quant. Biol., 51:263, (1987), Ehrlich (ed), PCR technology, Stockton Press, NY, 1989, Ehrlich et al, Science, 252:1643-1650, (1991), "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, Academic Press, New York, (1990).

Prior to any PCR that is to be performed, the complexity of a nucleic acid sample may be reduced where appropriate by creating a cDNA library for example using RT-PCR or by using the phenol emulsion reassociation technique (Clarke et al. (1992) NAR 20, 1289-1292) on a genomic library.

Thus a method involving use of PCR in obtaining nucleic acid according to the present invention may include:
(a) providing a preparation of plant nucleic acid,
(b) providing a pair of nucleic acid molecule primers useful in (i.e. suitable for) PCR, at least one said primers having a sequence shown in or complementary to a sequence shown in Seq ID No 1 as described in above,
(c) contacting nucleic acid in said preparation with said primers under conditions for performance of PCR,
(d) performing PCR and determining the presence or absence of an amplified PCR product. The presence of an amplified PCR product may indicate identification of a gene of interest or fragment thereof.

Thus the methods of the invention may include hybridisation of one or more (e.g. two) probes or primers to target nucleic acid. Where the nucleic acid is double-stranded DNA, hybridisation will generally be preceded by denaturation to produce single-stranded DNA. The hybridisation may be as part of a PCR procedure, or as part of a probing procedure not involving PCR. An example procedure would be a combination of PCR and low stringency hybridisation. A screening procedure, chosen

from the many available to those skilled in the art, is used to identify successful hybridisation events and isolated hybridised nucleic acid.

An oligonucleotide for use in probing or PCR may be about 30 or fewer nucleotides in length (e.g. 18, 21 or 24). Generally specific (i.e. "distinctive" or "characteristic") primers are upwards of 14 nucleotides in length. For optimum specificity and cost effectiveness, primers of 16-24 nucleotides in length may be preferred. Those skilled in the art are well versed in the design of primers for use processes such as PCR. If required, probing can be done with entire restriction fragments of the gene disclosed herein which may be 100's or even 1000's of nucleotides in length.

Some preferred oligonucleotides have a corresponding to bases 1 to 892 or 600 to 892 of Seq ID No 1. Primers may correspond to 1398 to 1417 or 2272 to 2290 of Seq ID No 1 (which is 1410-1429 and 2285-2302 on the afVP1 sequence deposisted on the EMBL database under accession number AFJ001140).

In a further (third) aspect of the present invention, the nucleic acid described above is in the form of a recombinant and preferably replicable vector.

"Vector" is defined to include, inter alia, any plasmid, cosmid, phage or *Agrobacterium* binary vector in double or single stranded linear or circular form which may or may not be self transmissible or mobilizable, and which can transform prokaryotic or eukaryotic host either by integration into the cellular genome or exist extrachromosomally (e.g. autonomous replicating plasmid with an origin of replication).

Specifically included are shuttle vectors by which is

meant a DNA vehicle capable, naturally or by design, of replication in both the actinomycetes and related species and in bacteria and/or eucaryotic (e.g. higher plant, mammalian, yeast or fungal cells).

A vector including nucleic acid according to the present invention need not include a promoter or other regulatory sequence, particularly if the vector is to be used to introduce the nucleic acid into cells for recombination into the genome.

However, in a preferred embodiment, the nucleic acid in the vector is under the control of (operably linked to) an appropriate promoter or other regulatory elements for expression in a host cell such as a microbial, e.g. bacterial, or plant cell. In the case of genomic DNA, this may contain its own promoter or other regulatory elements and in the case of cDNA this may be under the control of an appropriate promoter or other regulatory elements for expression in the host cell.

By "promoter" is meant a sequence of nucleotides from which transcription may be initiated of DNA operably linked downstream (i.e. in the 3' direction on the sense strand of double-stranded DNA).

"Operably linked" means joined as part of the same nucleic acid molecule, suitably positioned and oriented for transcription to be initiated from the promoter. DNA operably linked to a promoter is "under transcriptional initiation regulation" of the promoter.

Generally speaking, those skilled in the art are well able to construct vectors and design protocols for recombinant gene expression. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator

fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. For further details see, for example, *Molecular Cloning: a Laboratory Manual:* 2nd edition, Sambrook *et al*, 1989, Cold Spring Harbor Laboratory Press.

Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis (see above), sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in *Current Protocols in Molecular Biology*, Second Edition, Ausubel et al. eds., John Wiley & Sons, 1992. The disclosures of Sambrook et al. and Ausubel et al. are incorporated herein by reference. Specific procedures and vectors previously used with wide success upon plants are described by Bevan (Nucl. Acids Res. 12, 8711-8721 (1984)) and Guerineau and Mullineaux (1993) (Plant transformation and expression vectors. In: Plant Molecular Biology Labfax (Croy RRD ed) Oxford, BIOS Scientific Publishers, pp 121-148).

If desired, selectable genetic markers may be used consisting of chimaeric genes that confer selectable phenotypes such as resistance to antibiotics such as kanamycin, hygromycin, phosphinotricin, chlorsulfuron, methotrexate, gentamycin, spectinomycin, imidazolinones and glyphosate.

Thus this aspect of the invention provides a gene construct, preferably a replicable vector, comprising a promoter operatively linked to a nucleotide sequence provided by the present invention, such as the afVP1 gene shown in Seq ID No 1, a homologue thereof (e.g. taVP1), or any active mutant, derivative or allele thereof.

Suitable promoters include the Cauliflower Mosaic Virus

35S (CaMV 35S) gene promoter that is expressed at a high level in virtually all plant tissues (Benfey et al, 1990a and 1990b); the cauliflower meri 5 promoter that is expressed in the vegetative apical meristem as well as several well localised positions in the plant body, e.g. inner phloem, flower primordia, branching points in root and shoot (Medford, 1992; Medford et al, 1991) and the *Arabidopsis thaliana* LEAFY promoter that is expressed very early in flower development (Weigel et al, 1992).

Another strong promoter is the rice actin promoter. Also advantageous in the present context is the ubiquitin promoter which is expressed strongly in embryos (see Christenson & Quail (1996) Transgenic Research 5: 2133-2218.

Previous work in Arabidopsis has shown that constitutive expression of ABI 3 causes no negative effects on plant growth, and so expression of afVP 1 throughout a plant (e.g. wheat) may not have negative side effects on wheat plant growth.

However the promoter may include one or more sequence motifs or elements conferring developmental and/or tissue-specific regulatory control of expression. Other regulatory sequences may be included, for instance as identified by mutation or digest assay in an appropriate expression system or by sequence comparison with available information, e.g. using a computer to search on-line databases.

Thus in another embodiment of this aspect of the present invention, there is provided a gene construct, preferably a replicable vector, comprising an inducible promoter operably linked to a nucleotide sequence provided by the present invention, such as the afVP1 gene, a homolog from another plant species, e.g. a wheat taVP1, or any mutant,

or allele thereof.

The term "inducible" as applied to a promoter is well understood by those skilled in the art. In essence, expression under the control of an inducible promoter is "switched on" or increased in response to an applied stimulus. The nature of the stimulus varies between promoters. Some inducible promoters cause little or undetectable levels of expression (or no expression) in the absence of the appropriate stimulus. Other inducible promoters cause detectable constitutive expression in the absence of the stimulus. Whatever the level of expression is in the absence of the stimulus, expression from any inducible promoter is increased in the presence of the correct stimulus. The preferable situation is where the level of expression increases upon application of the relevant stimulus by an amount effective to alter a phenotypic characteristic. Thus an inducible (or "switchable") promoter may be used which causes a basic level of expression in the absence of the stimulus which level is too low to bring about a desired phenotype (and may in fact be zero). Upon application of the stimulus, expression is increased (or switched on) to a level which brings about the desired phenotype.

A suitable inducible promoter is the GST-II-27 gene promoter which has been shown to be induced by certain chemical compounds which can be applied to growing plants. The promoter is functional in both monocotyledons and dicotyledons. It can therefore be used to control gene expression in a variety of genetically modified plants, including field crops such as canola, sunflower, tobacco, sugarbeet, cotton; cereals such as wheat, barley, rice, maize, sorghum; fruit such as tomatoes, mangoes, peaches, apples, pears, strawberries, bananas, and melons; and vegetables such as carrot, lettuce, cabbage and onion. The GST-II-27

promoter is also suitable for use in a variety of tissues, including roots, leaves, stems and reproductive tissues.

Other advantageous promoters may be those which function at particular developmental stages (e.g. embryogenesis) - for instance the Em promoter, or the taVP1 wheat promoter which is discussed hereinafter.

In a fourth aspect the present invention also provides methods comprising introduction of such a construct into a plant cell and/or induction of expression of a construct within a plant cell, by application of a suitable stimulus, an effective exogenous inducer.

The vectors described above may be introduced into hosts by any appropriate method e.g. conjugation, mobilisation, transformation, transfection, transduction or electoporation.

However, when introducing a chosen gene construct into a cell, certain considerations must be taken into account, well known to those skilled in the art. The nucleic acid to be inserted should be assembled within a construct which contains effective regulatory elements which will drive transcription. There must be available a method of transporting the construct into the cell. Once the construct is within the cell membrane, integration into the endogenous chromosomal material either will or will not occur. Finally, as far as plants are concerned the target cell type must be such that cells can be regenerated into whole plants (see below).

Plants transformed with the DNA segment containing the sequence may be produced by standard techniques which are already known for the genetic manipulation of plants. DNA can be transformed into plant cells using any suitable

technology, such as a disarmed Ti-plasmid vector carried by Agrobacterium exploiting its natural gene transfer ability (EP-A-270355, EP-A-0116718, NAR 12(22) 8711 - 87215 1984), particle or microprojectile bombardment (US 5100792, EP-A-444882, EP-A-434616) microinjection (WO 92/09696, WO 94/00583, EP 331083, EP 175966, Green *et al.* (1987) *Plant Tissue and Cell Culture*, Academic Press), electroporation (EP 290395, WO 8706614 Gelvin Debeyser - see attached) other forms of direct DNA uptake (DE 4005152, WO 9012096, US 4684611), liposome mediated DNA uptake (e.g. Freeman *et al. Plant Cell Physiol.* 29: 1353 (1984)), or the vortexing method (e.g. Kindle, *PNAS U.S.A.* 87: 1228 (1990d) Physical methods for the transformation of plant cells are reviewed in Oard, 1991, *Biotech. Adv.* 9: 1-11.

Agrobacterium transformation is widely used by those skilled in the art to transform dicotyledonous species. Recently, there has been substantial progress towards the routine production of stable, fertile transgenic plants in almost all economically relevant monocot plants (Toriyama, et al. (1988) *Bio/Technology* 6, 1072-1074; Zhang, *et al.* (1988) *Plant Cell Rep.* 7, 379-384; Zhang, *et al.* (1988) *Theor Appl Genet* 76, 835-840; Shimamoto, *et al.* (1989) *Nature* 338, 274-276; Datta, *et al.* (1990) *Bio/Technology* 8, 736-740; Christou, *et al.* (1991) *Bio/Technology* 9, 957-962; Peng, *et al.* (1991) International Rice Research Institute, Manila, Philippines 563-574; Cao, *et al.* (1992) *Plant Cell Rep.* 11, 585-591; Li, *et al.* (1993) *Plant Cell Rep.* 12, 250-255; Rathore, *et al.* (1993) *Plant Molecular Biology* 21, 871-884; Fromm, *et al.* (1990) *Bio/Technology* 8, 833-839; Gordon-Kamm, *et al.* (1990) *Plant Cell* 2, 603-618; D'Halluin, *et al.* (1992) *Plant Cell* 4, 1495-1505; Walters, *et al.* (1992) *Plant Molecular Biology* 18, 189-200; Koziel, *et al.* (1993) *Biotechnology* 11, 194-200; Vasil, I. K. (1994) *Plant Molecular Biology* 25, 925-937;

Weeks, *et al.* (1993) *Plant Physiology* 102, 1077-1084; Somers, *et al.* (1992) *Bio/Technology* 10, 1589-1594; WO92/14828). In particular, *Agrobacterium* mediated transformation is now emerging also as an highly efficient alternative transformation method in monocots (Hiei *et al.* (1994) *The Plant Journal* 6, 271-282).

Particularly of interest on the present case is the fact that the generation of fertile transgenic plants has been achieved in the cereals rice, maize, wheat, oat, and barley (reviewed in Shimamoto, K. (1994) *Current Opinion in Biotechnology* 5, 158-162.; Vasil, *et al.* (1992) *Bio/Technology* 10, 667-674; Vain et al., 1995, *Biotechnology Advances* 13 (4): 653-671; Vasil, 1996, *Nature Biotechnology* 14 page 702).

Microprojectile bombardment, electroporation and direct DNA uptake are preferred where Agrobacterium is inefficient or ineffective. Alternatively, a combination of different techniques may be employed to enhance the efficiency of the transformation process, eg bombardment with Agrobacterium coated microparticles (EP-A-486234) or microprojectile bombardment to induce wounding followed by co-cultivation with Agrobacterium (EP-A-486233).

Following transformation, a plant may be regenerated, e.g. from single cells, callus tissue or leaf discs, as is standard in the art. Almost any plant can be entirely regenerated from cells, tissues and organs of the plant. Available techniques are reviewed in Vasil et al., *Cell Culture and Somatic Cell Genetics of Plants, Vol I, II and III, Laboratory Procedures and Their Applications*, Academic Press, 1984, and Weissbach and Weissbach, *Methods for Plant Molecular Biology*, Academic Press, 1989.

The particular choice of a transformation technology will

be determined by its efficiency to transform certain plant species as well as the experience and preference of the person practising the invention with a particular methodology of choice. It will be apparent to the skilled person that the particular choice of a transformation system to introduce nucleic acid into plant cells is not essential to or a limitation of the invention, nor is the choice of technique for plant regeneration.

Thus this aspect of the present invention includes a method of transforming a plant cell involving introduction of a vector comprising the afVP1 sequence (or a mutant or derivative thereof) into a plant cell and causing or allowing recombination between the vector and the plant cell genome to introduce the sequence of nucleotides into the genome.

In a fifth aspect of the invention, there is disclosed a host cell containing nucleic acid or a vector according to the present invention, especially a plant or a microbial cell.

The invention further encompasses a host cell transformed with nucleic acid or a vector according to the present invention, especially a plant or a microbial cell, and most preferably a crop plant e.g. wheat. Within the cell, the nucleic acid may be incorporated within the chromosome. There may be more than one heterologous nucleotide sequence per haploid genome.

Thus in one embodiment of the invention there is provided a plant cell having incorporated into its genome nucleic acid, particularly heterologous nucleic acid, as provided by the present invention, under operative control of a regulatory sequence for control of expression. The coding sequence may be operably linked to one or more regulatory sequences which may be heterologous or foreign

to the gene, such as not naturally associated with the gene for its expression. The nucleic acid according to the invention may be placed under the control of an externally inducible gene promoter to place expression under the control of the user.

The term "heterologous" is used broadly in this aspect to indicate that the gene/sequence of nucleotides in question have been introduced into said cells of the plant or an ancestor thereof, using genetic engineering, i.e. by human intervention. A heterologous gene (e.g. authentic afVP1) may replace an endogenous equivalent gene (e.g. taVP1; i.e. one which normally performs the same or a similar function) or the inserted sequence may be additional to the endogenous gene or other sequence. The heterologous (or exogenous or foreign) nucleic acid may be non-naturally occuring in cells of that type, variety or species. Thus the heterologous nucleic acid may comprise a coding sequence of or derived from a particular type of plant cell or species or variety of plant, placed within the context of a plant cell of a different type or species or variety of plant. A further possibility is for a nucleic acid sequence to be placed within a cell in which it or a homolog is found naturally, but wherein the nucleic acid sequence is linked and/or adjacent to nucleic acid which does not occur naturally within the cell, or cells of that type or species or variety of plant, such as operably linked to one or more regulatory sequences, such as a promoter sequence, for control of expression.

In the transgenic plant cell (i.e. transgenic for the nucleic acid in question) the transgene may be on an extra-genomic vector or incorporated, preferably stably, into the genome.

A plant may be regenerated from one or more transformed

plant cells described above. Such plants form a sixth aspect of the invention.

A plant according to the present invention may be one which does not breed true in one or more properties. Plant varieties may be excluded, particularly registrable plant varieties according to Plant Breeders' Rights. It is noted that a plant need not be considered a "plant variety" simply because it contains stably within its genome a transgene, introduced into a cell of the plant or an ancestor thereof.

In addition to a plant, the present invention provides any clone of such a plant, seed, selfed or hybrid progeny and descendants, and any part of any of these, such as cuttings, seed. The invention provides any plant propagule, that is any part which may be used in reproduction or propagation, sexual or asexual, including cuttings, seed and so on. Also encompassed by the invention is a plant which is a sexually or asexually propagated off-spring, clone or descendant of such a plant, or any part or propagule of said plant, off-spring, clone or descendant.

Particularly embraced are the seeds or grains of a transformed plant as described above, and also products (e.g. human and animal foodstuffs) derived from or containing such seeds or grains.

In a seventh aspect, the invention provides a method of influencing or affecting the dormancy characteristics of a plant, preferably the viviparous or PHS phenotype of a plant, including the step of causing or allowing expression of a heterologous nucleic acid sequence, as discussed in relation to the first and second aspects of the invention, within cells of the plant.

This aspect particularly provides a method of including expression from nucleic acid Seq ID No 1 or 2, or a mutant, allele or derivative of those sequences, within cells of a plant (thereby producing the encoded polypeptide), following an earlier step of introduction of the nucleic acid into a cell of the plant or an ancestor thereof. The method may employ the vectors of the third aspect.

In the present invention, expression (or over-expression or endogenous sequences) may be achieved by introduction of the nucleotide sequence in a "sense" orientation. Thus, the present invention provides a method of the method including causing or allowing expression of the product (polypeptide or nucleic acid transcript) encoded by heterologous nucleic acid according to the invention from that nucleic acid within cells of the plant.

The complete sequence corresponding to the coding sequence of afVP1 need not be used. For example fragments (i.e. active derivatives or mutants) of sufficient length may be used.

The sequence employed may be about 500 nucleotides or less, possibly about 400 nucleotides, about 300 nucleotides, about 200 nucleotides, or about 100 nucleotides. It may be possible to use oligonucleotides of much shorter lengths, 14-23 nucleotides, although longer fragments, and generally even longer than about 500 nucleotides are preferable where possible, such as longer than about 600 nucleotides, than about 700 nucleotides, than about 800 nucleotides, than about 1000 nucleotides or more.

Down-regulation of expression of a target gene (e.g. a homolog identified in accordance with the second aspect of the invention such as taVP1 - Seq ID No 2) may be

achieved using anti-sense technology or "sense regulation" ("co-suppression").

For instance, if it is desired to suppress dormancy (i.e. enhance PHS or 'malting') then the nucleic acids of the present invention (e.g. taVP1, afVP1, other derivatives) may be used for this purpose in accordance with standard procedures for anti-sense or sense suppression.

In using anti-sense genes or partial gene sequences to down-regulate gene expression, a nucleotide sequence is placed under the control of a promoter in a "reverse orientation" such that transcription yields RNA which is complementary to normal mRNA transcribed from the "sense" strand of the target gene. See, for example, Rothstein et al, 1987; Smith *et al*, (1988) *Nature* 334, 724-726; Zhang *et al*, (1992) *The Plant Cell* 4, 1575-1588, English *et al*., (1996) *The Plant Cell* 8, 179-188. Antisense technology is also reviewed in Bourque, (1995), *Plant Science* 105, 125-149, and Flavell, (1994) *PNAS USA* 91, 3490-3496.

An alternative is to use a copy of all or part of the target gene inserted in sense, that is the same, orientation as the target gene, to achieve reduction in expression of the target gene by co-suppression. See, for example, van der Krol *et al*., (1990) *The Plant Cell* 2, 291-299; Napoli *et al*., (1990) *The Plant Cell* 2, 279-289; Zhang *et al*., (1992) *The Plant Cell* 4, 1575-1588, and US-A-5,231,020. Recent work indicates that foreign (non-endogenous) homologous sequences may be particularly effective at inducing gene silencing in targeted endogenous genes. See e.g. Matzke, M. A. and Matzke, A. J. M. (1995), *Trends in Genetics*, 11: 1-3). This sequence homology may involve promoter regions or coding regions of the silenced gene (Matzke, M. A. and Matzke, A. J. M. (1993) *Annu. Rev. Plant Physiol. Plant Mol.*

Biol., 44: 53-76, Vaucheret, H. (1993) *C. R. Acad. Sci. Paris*, 316: 1471-1483, Vaucheret, H. (1994), *C. R. Acad. Sci. Paris*, 317: 310-323, Baulcombe, D. C. and English, J. J. (1996), Current Opinion In Biotechnology, 7: 173-180, Park, Y-D., et al (1996), *Plant J.*, 9: 183-194.

Thus the sequences of the present invention may have utility when used in plant species different to those from which they were derived (e.g. barley).

In an eight aspect, the present invention also encompasses the expression product of any of the nucleic acid sequences disclosed, particularly those of the first and second aspects, and methods of making the expression product by expression from encoding nucleic acid therefore under suitable conditions, which may be in suitable host cells.

Following expression, the product may be isolated from the expression system (e.g. microbial) and may be used as desired, for instance in formulation of a composition including at least one additional component.

Alternatively (and indeed preferably) the product may perform its function *in vivo*, in this context the function being to influence the dormancy characteristics of a plant, preferably the viviparous or PHS phenotype of a plant.

In an ninth aspect, purified or semi-purified afVP1 or taVP1 protein, or a fragment, mutant, derivative or other variant thereof, e.g. produced recombinantly by expression from encoding nucleic acid therefor, may be used to raise antibodies employing techniques which are standard in the art. Antibodies and polypeptides comprising antigen-binding fragments of antibodies may be used in identifying homologs from other species as discussed further below, and also in labelling proteins.

Methods of producing antibodies include immunising a mammal (e.g. human, mouse, rat, rabbit, horse, goat, sheep or monkey) with the protein or a fragment thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and might be screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al, 1992, Nature 357: 80-82). Antibodies may be polyclonal or monoclonal. As an alternative or supplement to immunising a mammal, antibodies with appropriate binding specificity may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces; for instance see WO92/01047.

Antibodies may have utility in testing for endogenous VP1-analog expression (especially taVP1 expression in wheat) as part of a dormancy/PHS assessment, as is discussed hereinafter.

Antibodies raised to a polypeptide or peptide can be used in the identification and/or isolation of homologous polypeptides, and then their encoding genes.

Thus, the present invention provides a method of identifying or isolating a polypeptide having one or more afVP1 (or taVP1) epitopes comprising screening candidate polypeptides with a polypeptide comprising the antigen-binding domain of an antibody (for example whole antibody or a fragment thereof) which is able to bind afVP1 (or taVP1) or a fragment or a derivative thereof or preferably has binding specificity for such a polypeptide.

Thus specific binding members such as antibodies and polypeptides comprising antigen binding domains of antibodies that bind and are preferably specific for afVP1 or taVP1 polypeptides or mutants or derivatives thereof represent part of the present invention, as do their use and methods which employ them.

Candidate polypeptides for screening may for instance be the products of an expression library created using nucleic acid derived from an plant of interest, or may be the product of a purification process from a natural source. A polypeptide found to bind the antibody may be isolated and then may be subject to amino acid sequencing. Any suitable technique may be used to sequence the polypeptide either wholly or partially (for instance a fragment of the polypeptide may be sequenced). Amino acid sequence information may be used in obtaining nucleic acid encoding the polypeptide, for instance by designing one or more oligonucleotides (e.g. a degenerate pool of oligonucleotides) for use as probes or primers in hybridization to candidate nucleic acid, or by searching computer sequence databases, as discussed further below.

The above description has generally been concerned with the coding parts of the afVP1 gene, and uses therefor. Also embraced within the present invention are untranscribed parts of that gene, or the taVP1 gene.

Thus a tenth aspect of the invention is a nucleic acid molecule encoding the promoter of the afVP1 gene or the taVP1 gene.

The promoter region may be readily identified using a probe or primer based on Seq ID No 1 or Seq ID no 2, as described in relation to earlier aspects. This can be used in the identification and isolation of a promoter from a genomic library containing DNA derived from a

plant source. Techniques and conditions for such probing are well known in the art as is discussed above. Following probing, promoter activity is assessed using a test transcription system.

"Promoter activity" is used to refer to ability to initiate transcription. The level of promoter activity is quantifiable for instance by assessment of the amount of mRNA produced by transcription from the promoter or by assessment of the amount of protein product produced by translation of mRNA produced by transcription from the promoter. The amount of a specific mRNA present in an expression system may be determined for example using specific oligonucleotides which are able to hybridise with the mRNA and which are labelled or may be used in a specific amplification reaction such as the polymerase chain reaction.

Use of a reporter gene facilitates determination of promoter activity by reference to protein production. The reporter gene preferably encodes an enzyme which catalyses a reaction which produces a detectable signal, preferably a visually detectable signal, such as a coloured product. Many examples are known, including β-galactosidase and luciferase. β-galactosidase activity may be assayed by production of blue colour on substrate, the assay being by eye or by use of a spectro-photometer to measure absorbance. Fluorescence, for example that produced as a result of luciferase activity, may be quantitated using a spectrophotometer. Radioactive assays may be used, for instance using chloramphenicol acetyltransferase, which may also be used in non-radioactive assays. The presence and/or amount of gene product resulting from expression from the reporter gene may be determined using a molecule able to bind the product, such as an antibody or fragment thereof. The binding molecule may be labelled directly or indirectly

using any standard technique.

Those skilled in the art are well aware of a multitude of possible reporter genes and assay techniques which may be used to determine promoter activity. Any suitable reporter/assay may be used and it should be appreciated that no particular choice is essential to or a limitation of the present invention.

Also embraced by the present invention is a promoter which is a mutant, derivative, or other homolog of the promoter identified as above. These can be generated or identified in similar manner to the derivatives discussed in the second aspect; they will share homology with the taVP1 or afVP1 promoters and retain promoter activity.

To find minimal elements or motifs responsible for tissue and/or developmental regulation, restriction enzyme or nucleases may be used to digest a nucleic acid molecule, or mutagenesis may be employed, followed by an appropriate assay (for example using a reporter gene such as luciferase) to determine the sequence required. Nucleic acid comprising these elements or motifs forms one part of the present invention.

In an eleventh aspect of the invention there is provided a nucleic acid construct, preferably an expression vector, including a promoter region or fragment, mutant, derivative or other homolog or variant thereof able to promote transcription as discussed above, operably linked to a heterologous gene, e.g. a coding sequence, which is preferably not the coding sequence with which the promoter is operably linked in nature.

The above aspects of the invention are concerned generally with methods and materials which have utility, *inter alia*, in manipulating PHS and/or other dormancy

related traits (secondary and after-ripening) in crops, particularly wheat, by means of transformation.

However the identification of the wheat homolog taVP1 by the present inventors has also opened up the possibility of improved methods for generating plants having desirable characteristics as regards PHS and/or other dormancy related traits. These methods have as their basis the identification and molecular tagging of the taVP1 gene (using taVP1 cDNA as a probe in genomic southern blots) which has been achieved by the present inventors, as described below.

A plant breeding approach to improving the PHS properties of wheat has a number of advantages over transformation approaches, particularly as regards consumer confidence in the improved products and ease of regulatory approval.

Essentially, plant breeding is the process of bringing together new combinations of genes, from different parents, allowing them to reassort into recombinant genotypes carrying various mixtures of the original parental genes, then selecting individual progenies which carry genetic combinations superior to the original parents.

Dormancy is one of a number of characteristics described as "quantitative traits" (i.e. varies over a continuous range as opposed to a trait such as grain colour which is an "all or nothing", discontinuous character).

Quantitative traits (= QTs) are controlled by several/many genes (situated on the chromosomes at several/many Quantitative Trait Loci = QTLs); and by "environmental" variables including weather effects and experimental uncertainties of measurement. Breeding for desirable QTs thus demands the ability to effectively

discriminate between genetic variants at a large number of QTLs, conventionally by using statistical techniques based on large samples and repeated trials.

Dormancy is a particularly difficult QT for the plant breeder because the effects of "error" variables are comparatively large, the trait itself is lost gradually during after-ripening, and experimental methods for testing dormancy are time/labour/material intensive. Since dormancy tests have to be carried out between one harvest and the next sowing (sometimes just a month or so), only limited time is available for empirical testing; for this reason wheat breeders usually defer dormancy testing until the later stages of a breeding programme when only a limited number of "elite" progenies have passed through earlier rounds of selection for other, more easily selectable characters. This has the effect of reducing the amount of control which the breeder can exercise over the genetic combinations of dormancy QTLs passing into his new varieties.

When selecting progeny, rather than use "direct" selection for dormancy (i.e. by measuring the trait itself), in which genetic combinations are unconsciously chosen on the basis of their statistical performance in empirical tests, it is possible to exploit "marker aided" selection, choosing progenies on the basis of discontinuous traits, each of which is simply controlled by genetic variants at a single (or small number) of genetic locus/loci. The conventional marker for dormancy in wheat is grain colour; it has been known for many years that red-seeded wheats tend to be more dormant than white-seeded wheats, and that grain colour is determined by dominant "red" versus recessive "white" alleles (gene variants) at three major gene loci. By discarding any white-seeded progenies (detected by visual inspection after steeping grains in dilute aqueous sodium hydroxide)

wheat breeders aim to eliminate the undesirable lack of dormancy associated with this character.

Thus one approach to breeding for PHS resistance is disclosed by Derera NF (1989) in "Breeding for pre-harvest sprouting tolerance". pp111-128 in: NF Derera (Ed), "Pre-Harvest Field Sprouting in Cereals. CRC Press Inc." This gives some specific examples of breeding programmes which have been undertaken to produce new varieties with improved dormancy, including exploitation of red grain colour, breeding for dormancy in white-grained wheats, screening wheat genotypes for use as donors of dormancy genes in breeding, and a suggested scheme for dormancy breeding. The use of the Hagberg falling number test (to assess the viscosity of ground kernel material under moist and dry conditions) is also discussed as being a useful measure of sprouting resistance.

Another discussion of PHS is found in Mares DJ (1989) "Pre-harvest sprouting damage and sprouting tolerance: assay methods and instrumentation." pp129-170 in the same volume as Derera (supra). This disclosed various methods used to select against sprouting. The Hagberg test, and its approval by various standardisation bodies, is also discussed. It is noted that an absence of sprouting damage (indicative of low PHS susceptibility) leads to high falling numbers (>400, generally 450-550).

The role of grain colour in dormancy, and its relationship with VP1, has been discussed in number of prior art papers. For instance the poster by Wilson (described in the prior art section above) discussed the possible orthology of the Red grain locus in wheat to the maize *VP-1* locus. Similarly Sorrells & Wilson (1997) Crop Science 37: 691-697 discusses the relationship between maize red pericarp colour (controlled by the P1 gene) nd

VP1, and suggests that a (postulated) wheat VP1 homolog may express via a P homolog.

Unfortunately the association between grain redness and dormancy is not hard and fast: breeders of white-grained wheats (eg. Australia) or amber wheats (eg. durum) must resort to other QTLs, also it has been noted that red wheats vary widely in dormancy. The most dormant white wheats can be as dormant as the least dormant reds: within both colour groups there is a continuous spectrum of dormancy, the scores of red wheats are shifted to the more dormant end of the spectrum, but there is considerable overlap between the two groups. This makes it clear that grain colour is not the sole determinant of dormancy.

Thus it can be seen that further markers to assist in the assessment of PHS resistance and other dormancy-related traits would be beneficial in breeding improved cultivars.

Ideally such markers should be in as close genetic linkage with the QTL as possible, or even better that it have a direct effect on the QT (reducing or even better eliminating the possibility of recombination). Selecting particular alleles which are known to directly exert particular phentoypic effects is termed Direct Allele Selection ('DAS'). Additionally the QTL which is marked should be an important determinant of the QT score. Finally it should also be practicable. Molecular markers, which depend on variation (polymorphisms) in the sequences of bases, are particularly useful in this regard.

The present inventors have now mapped the chromosomal location of the novel *taVp1* genes which they isolated.

Briefly, an RFLP polymorphism was identified between two parents of an F2 mapping population, then the alleles present in each individual of the population were determined. These genotypes at the *taVp1* locus were then compared with the genotypes of the same individuals at other, genetically linked loci determined previously (Devos et al 1992). Two of the three *taVp1* loci were mapped in this way (one on chromosome 3A, one on 3D); the third locus was detected on chromosome 3B by nullisomic analysis.

As with the grain colour genes, *taVp1* copies reside at three loci on the long arms of chromosomes 3A, 3B and 3D respectively. The results were compared to the consensus wheat map of Gale et al 1995 [(M.D.Gale, M.D.Atkinson, C.N.Chinoy, R.L.Harcourt, J.Jia, Q.Y.Li & K.M.Devos. 1995. Genetic maps of hexaploid wheat. pp29-40 in: Proceedings 8th International Wheat Genetics Symposium, Eds Z.S.Li & Z.Y.Xin, China Agricultural Scientech Press, Beijing] and homologous *taVp1* loci were assigned to the interval between loci *Xwg110* and *Xpsr549* on the consensus map (see Figure 7).

Interestingly it was found that the *taVp1* genes are linked to the *R* (colour genes) only at a distance of about 25 centiMorgans. This is clear evidence that *taVp1* and *R* genes are different, so that both marker systems can be used for manipulating dormancy, either in concert or independently. Both markers have direct effects, so loss of effect due to recombination is not a problem. The linkage interval between the two loci on each chromosome is large enough to allow a practicably high frequency of recovery of *taVp1/R* recombinants should these be required (eg. any existing linkage between a "good" *taVp1* allele and a "red" colour allele could realistically be broken for introduction of the *taVp1*-based dormancy into white wheats).

Apart from the option for breeding dormant white/amber wheats, *taVp1* offers advantages over use of the colour marker arising from the availability of clones of wheat alleles. The problems with the colour marker are that it cannot be scored until the grain is ripe, and that no information is available about which of the *R* genes is present without lengthy and large-scale breeding experiments. Knowledge of the *taVp1* DNA, RNA, and/or protein sequences allows the identification of individual alleles present in a sample of tissue, e.g. DNA from the first seedling leaf, or even taken from a seed prior to sowing.

Polymorphisms can be manifest in a number of ways. Structurally they will alter the characteristics of the DNA to bind probes and primers at particular sites, or its properties as a substrate for restriction analysis. Functionally they may affect the quality or quantity of mRNA or protein product which derives from the DNA. Thus, for instance, the presence of absence of a lesion in a promoter or other regulatory sequence may be assessed by determining the level of mRNA production by transcription or the level of polypeptide production by translation from the mRNA. The level of mRNA or protein will be affected not only by its rate of production, but also by its stability and rate of degradation.

Thus the sequence information (nucleic and/or protein product) disclosed herein enables the use of specific amplification, probing or other techniques, to carry out allele identification and hence germplasm classification.

"Nucleic acid sequence" in this context embraces the coding sequences, introns, and promoters of the relevant allele, plus also post-transcriptional modifications of RNA. Thus tests may be carried out on preparations containing genomic DNA, cDNA and/or mRNA. Testing cDNA

or mRNA has the advantage of the complexity of the nucleic acid being reduced by the absence of intron sequences, but the possible disadvantage of extra time and effort being required in making the preparations. RNA may be more difficult to manipulate than DNA because of the wide-spread occurrence of RN'ases.

"Protein sequence" in this context covers both the primary structure, plus post-translational protein modifications. Under certain circumstances the total absence of a detectable protein product will be indicative of alterations in the encoded protein sequence.

Generally the methods may make use of biological samples from one or more plants or cells (e.g. in a seed) that are suspected to contain the nucleic acid sequences or polypeptide.

The following method are exemplary only. Those skilled in the art will appreciate that other methods which may be devised without burden on the basis of the information made available by the present inventors also form part of the present invention. For instance a number of methods for determining the presence and identity of polymorphic molecular markers (in the context of biodiversity analysis) are disclosed by Karp et al (1997) Biotechnology 15: 625-628. Such methods may have analagous utility in carrying out the present invention.

1) At the nucleic acid level, identification may involve hybridisation of a suitable specific oligo- or poly-nucleotide probe, such as a fragment of those disclosed herein, or further allelic sequences established using the information disclosed herein. Where the nucleic acid target is double-stranded DNA, hybridisation will generally be preceded by denaturation to produce single-

stranded DNA. Such methods include Southern and Northern hybridisations, which can be used both qualitatively or quantitively (e.g. to assess mRNA level). A screening procedure, chosen from the many available to those skilled in the art, may be used to identify successful hybridisation events and isolate hybridised nucleic acid. For instance, probes may be radioactively, fluorescently or enzymatically labelled.

Preferably the screening is carried out with a variant- or allele-specific probe - this is particular useful for DAS. Such a probe corresponds in sequence to a region of the gene, or its complement, containing a sequence alteration known to be associated with the trait of interest. Under suitably stringent conditions, specific hybridisation of such a probe to test nucleic acid is indicative of the presence of the sequence alteration in the test nucleic acid. For efficient screening purposes, more than one probe may be used on the same test sample.

When screening for particular alleles, the nucleic acid in the sample may initially be amplified, e.g. using PCR, to increase the amount of the analyte as compared to other sequences present in the sample. This allows the target sequences to be detected with a high degree of sensitivity if they are present in the sample. This initial step may be avoided by using highly sensitive array techniques

Approaches which rely on hybridisation between a probe and test nucleic acid and subsequent detection of a mismatch may be employed. Under appropriate conditions (temperature, pH etc.), an oligonucleotide probe will hybridise with a sequence which is not entirely complementary. The degree of base-pairing between the two molecules will be sufficient for them to anneal despite a mis-match. Various approaches are well known

in the art for detecting the presence of a mis-match between two annealing nucleic acid molecules. For instance, RN'ase A cleaves at the site of a mis-match. Cleavage can be detected by electrophoresing test nucleic acid to which the relevant probe or probe has annealed and looking for smaller molecules (i.e. molecules with higher electrophoretic mobility) than the full length probe/test hybrid. Other approaches rely on the use of enzymes such as resolvases or endonucleases.

Thus, an oligonucleotide probe that has the sequence of a region of the normal gene (either sense or anti-sense strand) in which polymorphisms associated with the trait of interest are known to occur may be annealed to test nucleic acid and the presence or absence of a mis-match determined. Detection of the presence of a mis-match may indicate the presence in the test nucleic acid of a mutation associated with the trait. On the other hand, an oligonucleotide probe that has the sequence of a region of the gene including a mutation associated with disease resistance may be annealed to test nucleic acid and the presence or absence of a mis-match determined. The presence of a mis-match may indicate that the nucleic acid in the test sample has the normal sequence, or a different mutant or allele sequence. In either case, a battery of probes to different regions of the gene may be employed.

(ii) Allele- or variant- (or even genome-) specific oligonucleotides may similarly be used in PCR to specifically amplify particular sequences if present in a test sample. Assessment of whether a PCR band contains a gene variant may be carried out in a number of ways familiar to those skilled in the art.

The PCR product may for instance be treated in a way that enables one to display the mutation or polymorphism on a denaturing polyacrylamide DNA sequencing gel, with

specific bands that are linked to the gene variants being selected. It may also be desirable to analyse DNA fragment size, restriction site variation (e.g. CAPS - cleaved amplified polymorphic sites) and so on. Sequence Tagged Site (STS) Polymerase Chain Reaction (PCR) is rapid, specific, and does not require use of radiosotopes/autoradiography.

By way of Examples, the following primers could be used to distinguish the certain alleles disclosed in Figures 6 and 8. Further primer combinations can be devised without burden by those skilled in the art for new taVP1 alleles if and when they are identified (for instance by use of the materials and methods disclosed herein).

Non specific primer (reverse compliment):

Position 2210/clone 10: CGT CAC ATC TGA CCG ATA GC

Primers to differentiate clones 6+9 from 5+10:

6 + 9 specific: position 1696: CAT CTC AGG TGT GGA GCA TGC

5 + 10 specific: position 1691: CGG CAC ATC TCA GAT TTT GGC CC

Primers to differentiate clones 5+6 from 9+10:

5+6 specific: position 1432: GCG GCA GCA GGG TGC GAG G
9+10 specific: position 1432:GCG GCA GCA GGT GCA TGC ATG

Further primers which are specific for the A- B- and D-genomes are discussed in the Examples hereinafter.

(iii) RFLP, hybridized to homologous or heterologous probes based on the sequences disclosed herein. The

presence of differences in sequence of nucleic acid molecules may be detected by means of restriction enzyme digestion, such as in a method of DNA fingerprinting where the restriction pattern produced when one or more restriction enzymes (chosen on the basis of the sequences disclosed herein) are used to cut a sample of nucleic acid is compared with the pattern obtained when a sample containing the desired allele or a variant digested with the same enzyme or enzymes. Amplified Fragment Length Polymorphism (AFLP) can be carried out using primers devised on the basis of the sequences disclosed herein. The strength of AFLP is the ability to screen a large number of different marker molecules in a single test. Analysis of the products can be carried out using e.g. by gel electrophoresis, capillary electrophoresis.

(iv) RT-PCR is based on the amplification of RNA, which may be qualitative or quantitative. Essentially this method uses reverse transcriptase to generate a DNA copy of plant mRNA.

(v) Variations in antibody/antigen complexes between plant protein extracts and antibodies targeted to protein epitopes. This is particular useful for distinguishing functional from non-functional proteins (e.g. truncated forms, wherein a particular allele contains a premature stop codon). Thus a sample may be tested for the presence of a binding partner for an antibody (or mixture of antibodies), specific for one or more allelic variants. Binding is assessed by any means commonly known to those skilled in the art. Where a panel of antibodies is used, different reporting labels may be employed for each antibody so that binding of each can be determined.

A specific binding member such as an antibody may be used to isolate and/or purify its binding partner polypeptide from a test sample, to allow for sequence and/or

biochemical analysis of the polypeptide to determine whether it has the sequence and/or properties of the wild-type polypeptide or a particular mutant, variant or allele thereof. Amino acid sequence is routine in the art using automated sequencing machines.

Thus the work of the present inventors offers a completely novel approach for manipulating seed dormancy in breeding programmes, exploiting the *taVp1* gene sequence which was previously unknown for direct selection of alleles controlling a QT for which currently available techniques are laborious, slow, and inefficient.

Various aspects of the invention will now be discussed in more detail:

In one aspect of the invention there is disclosed a method for assessing the PHS and/or other dormancy related properties of a wheat plant, the method comprising use of the molecular marker taVP1 which occurs in the interval between loci *Xwg110* and *Xpsr549* on the wheat group 3 consensus map.

In a further aspect of the invention these is disclosed of producing a cultivar comprising the steps of selecting a parent line having desired PHS and/or other dormancy related properties, breeding with that line, and selecting progeny on the basis of the molecular marker taVP1 described above.

Preferably the selection of the parent line(s) and/or progeny is done on the basis of specific superior alleles (i.e. DAS). This allows precise manipulation of variation of PHS via selection of progenies with appropriate, desired functional activity of characterised alleles. Thus PHS may be improved by selection for high levels of

expression of fully-functional alleles, firstly by selecting parents carrying desirable genomic copies of taVp1 gene(s), then by selecting progeny which express these alleles strongly at appropriate stages of seed development and maturation.

The assessment can be on the basis of analysing taVP1 DNA, RNA or protein as described above, and then correlating the result of the analysis with the expected PHS phenotype.

Parent plants possessing favoured alleles may be obtained from within an existing variety genepool, or prepared mutants from within an elite genepool.

Alternatively desirable alleles or may also be detected and transferred from one or more of the many wild or cultivated relatives of the plant, for which established methods are available for the introduction of "alien" variation into the plant genome. The correlation of the PHS trait with the afVP1 sequence greatly facilitates the identification and selection of desirable alleles in exotic germplasm. This is especially useful for species in which genetic variation in cultivated germplasm is limited (e.g. wheat *T aestivum* - see Chao et al (1989) Theor Appl Genet 88:717-721).

In the past, using traditional methods, problems with using exotic germplasm have included the low frequency of desirable alleles, and difficulties with linkage drag and polygenic inheritance. Such problems will be minimised by use of the present invention.

Lines may be produced by breeding from selected lines in accordance with standard techniques well known to those skilled in the art.

Clearly the PHS phenotype can be manipulated up or down; for applications in which dormancy is undesirable, e.g. malting, the same information and techniques could be employed to select in the reverse direction, i.e. to fix defective or poorly expressed copies of taVP1.

The demonstration by the present inventors that the afVP1 and seed colour gene alleles are linked but separate demonstrates that both marker systems can be used for manipulating dormancy, either in concert or independently. Thus methods of selection based on taVP1 alone (e.g. in white/amber grained wheats) or both taVP1 and red grain colour (e.g. in red wheats) also form part of the present invention.

Methods in which the taVP1 allele in particular plant or line is assessed and the result of the assessment is correlated directly with expected PHS phenotype, e.g. for the purposes of timing harvest, form a further part of the invention.

The taVP1 assessment may also be used to assess pedigree or phylogenetic origin if desired.

Nucleic acid-based determination of the identity of a particular *taVP1* allele (e.g. as in the methods described above) may be combined with determination of the genotype of the flanking linked genomic DNA and other unlinked genomic DNA using established sets of markers such as RFLPs, microsatellites or SSRs, AFLPs, RAPDs etc. This enables the researcher or plant breeder to select for not only the presence of the desirable *taVP1* allele but also for individual plant or families of plants which have the most desirable combinations of linked and unlinked genetic background. Such recombinations of desirable material may occur only rarely within a given segregating breeding population or backcross progeny. Direct assay

of the *taVP1* locus as afforded by the present invention allows the researcher to make a stepwise approach to fixing (making homozygous) the desired combination of flanking markers and taVP1 alleles, by first identifying individuals fixed for one flanking marker and then identifying progeny fixed on the other side of the locus all the time knowing with confidence that the desirable *taVP1* allele is still present.

The sequence information provided herein also allows the design of diagnostic tests and kits for determination of the presence of particular taVP1 and afVP1 alleles, in any given plant, cultivar, variety, population, landrace, part of a family or other selection in a breeding programme or other such genotype. A diagnostic test may be based on determination of the presence or absence of a particular allele by means of nucleic acid or polypeptide determination.

Plants which are generated (or assessed and or approved) using the taVP1-allele assessment methods of the present invention form a further aspect of the invention. Plants in this context embraces cultivars, and seeds, microspores, protoplasts, cotyledons, zygotes (ovules, pollen) and vegetative parts derived therefrom. It further embraces any clone of such a plant, selfed or hybrid progeny and descendants, and any part of any of these, such as cuttings and seed. Products made from such plants e.g. milled or malted grains, flour etc. are also embraced by the invention.

The invention will now be further illustrated with reference to the following non-limiting Figures and Examples. Other embodiments falling within the scope of the invention will occur to those skilled in the art in the light of these.

SEQUENCE ID NOS

Seq ID No 1: (see Figure 4(a)) "afVP1" cDNA sequence.

Seq ID No 2: (See Figure 10(a)): "taVP1" cDNA sequence.

FIGURES

Figure 1. Germination behaviour of seed from inbred lines of *Avena fatua*.

Figure 2. Northern blot analysis of gene expression patterns of imbibed seed from inbred lines subjected to different environmental conditions. a. Freshly harvested seed, b. Freshly harvested and after-ripened seed, c. Seed stored at 4°C or 24°C for one year, d. Seed following induction of secondary dormancy (Treated) or Untreated. In each case RNA was extracted from seed following 48 h imbibition. RNA loadings for each sample were 3μg and 0.5μg polyA containing RNA per lane. % germination (G%) of seed at the time of RNA extraction is indicated.

Figure 3. Northern blot analysis shows *afVP* 1 expression in the dry seed is positively correlated to length of after-ripening time required to break dormancy. Germination percentages [G(%)] are shown for four inbred lines 1, 3 and 6 months after harvest. RNA loadings for each sample were 3μg and 0.5μg polyA containing RNA per lane.

Figure 4(a): Seq ID No 1, "afVP1" cDNA sequence.

Figure 4(b): Comparison of afVP 1 predicted protein sequence with other VP 1 like transcription factors. Comparison of the protein sequences from *Avena fatua* (afVP 1), maize (VP 1), rice (osVP 1), bean (ALF 1),

Arabidopsis (ABI 3).

Figure 5. Model for the control of embryo dormancy and *afVP* 1 RNA levels by a switch that shows properties of reversibility.

Figure 6. taVP1 allele (clone 4).

Figure 7: Locations of orthologues in wheat (*taVp1*), rice (*osVP1*) and maize (*VP1*). From left to right the rice maps are based on Kurata et al (1994a,b) and Quarrie et al (1997), wheat on the concensus map of Gale et al (1995), maize (inverted relative to wheat and rice) on the BNL'95 (Anon, 1995) and rice on the map of Causse et al (1994) with additional markers mapped by McCough et al (1996) and the centromeres positioned proximal to *cdo920* as demonstrated by Singh et al (1996). Homeologous marker loci present on two or more maps are joined by dotted lines. Arrows indicate centromeres. Figures to the left of each map denote intervals in centiMorgans.

Figure 8. Clone 10, representing the taVP1 cDNA sequence. Also shown are further taVP1 alleles (clones 5, 6 and 9) which have been sequenced to various degrees.

Figure 9. Northern blot analysis of transgenic wheat plants containing afVP1 sequence.

Figure 10(a): Seq ID No 2, "taVP1" cDNA (clone 10)
Figure 10(b): clone 2
Figure 10(c): clone 3
Figure 10(d): clone 4
Figure 10(e): clone 5
Figure 10(f): clone 6
Figure 10(g): clone 9

Figure 11. Alignment of sections of six taVP1 clones

demonstrating that they fall into two groups (group I = 4, 5, 10; group II = 3, 6, 9).

Figure 12. Results of PCR using templates specific for the two groups. The results using primer set 4 suggest that clone 4 is found on chromosome 3A. The results using primer set 9 suggest that clone 9 is found on chromosome 3D.

EXAMPLES

EXAMPLE 1 - Analysis of role of afVP1 in dormancy and after-ripening

Plant material;

A. fatua inbred lines CS40, AN127, AN51 SH99 used in this study were obtained from Professor G Simpson (Univ. Of Saskatchewan, Canada). The lines were derived from single seeds selfed for six generations and grown in controlled environment rooms (Jana *et al.* 1988). Inbred line Bampton has been described previously (Hooley *et al.* 1991, Rushton *et al.* 1992) derived from single seed selfed for at least 10 generations, inbred line Rewe (Peters 1991) was derived from single seed selfed for 3 generations. Mature seeds were obtained from plants grown outdoors during the summer each year. Air dry seeds were stored in the dark in constant environment chambers at 15 °C unless otherwise stated. After-ripening and dormancy levels were monitored every three months by a germination assay.

Germination assays;

Germination assays were conducted at 22°C in the dark. Seeds were first de-husked, surface-sterilized with 10% (v/v) Parozone (Jeyes Ltd, Norfolk, UK) for 10 minutes, washed with sterile water, and then incubated embryo-side

up on moist sterile glass fibre paper for the times indicated in individual figures.

Results:

Six inbred lines of *A. fatua* were used to study the relationship between genetics and environment in the control of embryo dormancy. Dormancy/germination potential of seeds from these lines was assessed over a twelve month period from harvest, following storage at 24°C. The inbred line CS40 showed an extremely non-dormant phenotype. Dry seeds stored at 24°C after-ripened within 1 month and embryos germinated within 48 h of imbibition (figure 1a). Lines AN51 and SH99 showed higher levels of primary dormancy than CS40, although this dormancy rapidly diminished with after-ripening. Line AN127, Rewe and Bampton showed a greater degree of dormancy. Embryos from these lines after-ripened slowly, taking between 6 months to 1 year to lose dormancy when stored at 24 °C. During the period of after-ripening the time taken between initiation of imbibition and germination was much longer in lines showing dormancy, than in CS40. However this lag time decreased as after-ripening time increased and dormancy was lost.

Primary dormancy in Bampton was high (figure 1a), but after 3 years storage at 24°C dormancy was lost (indicating that after-ripening was completed). However, embryos from seeds stored at 4°C for the same period were still completely dormant (figure 1b), showing that temperature is an important determinant of the time required for after-ripening. Dormancy of Bampton seeds stored at 4°C could only be broken following imbibition at 24°C by a combination of GA treatment and mechanical rupture (data not shown).

The effects of conditions that induce secondary dormancy were investigated in three lines, CS40 (non-dormant, ND),

AN127 and Bampton (both dormant, D) following complete after-ripening of the embryos (figure 1 c). Secondary dormancy was induced by immersing seeds in de-gassed water for 70 h at 24°C in the dark. Embryos were then tested for dormancy potential in the normal germination assay. Final germination levels of seeds of the line CS40 were unaffected by the inductive treatment, although germination was slightly delayed. However seeds of AN127 and Bampton were highly susceptible to the treatment, showing reversion to a dormant phenotype following imbibition.

Extraction and analysis of RNA;

Poly-A containing RNA was extracted from seeds as described previously (Grierson 1992). RNA was size fractionated on 1.5% agarose MOPS-formaldehyde gels and transferred to a nitrocellulose membrane (Sambrook *et al.* 1989). Specific mRNAs were detected by hybridisation to maize VP1 cDNA clones from McCarty et al (1989) The Plant Cell 1, 523-532, labelled with α-^{32}P[dCTP] by random-priming using the Stratagene Prime-it II kit according to the manufacturers recommendations. Hybridisation conditions were 50% formamide, 6xSSPE, 5x Denhardts, 0.5% SDS, 100μg/ml denatured calf thymus DNA, at 42°C for 16 h. Filters were washed once for 10 min at room temperature in 1xSSPE, 0.1% SDS, once for 30 min at room temperature in 1xSSPE, 0.1% SDS, and finally 30 min at 55°C in 1xSSPE, 0.1% SDS (Sambrook *et al.* 1989). Prior to autoradiography X-ray film was pre-flashed to ensure detected signals were within the linear range of detection for the film.

Results:

We analysed gene expression patterns in seeds to determine whether the expression of specific genes was

regulated, by genetic background and environment, in the same way as dormancy/germination phenotypes in the inbred lines of *A. fatua*. Two marker genes were chosen that have previously been linked to particular developmental states of embryos (*AMY* 2/1 and *Em*) (table 1). Other genes analysed were *af* 10 (expressed throughout development), and the *A. fatua* homologue of the maize transcription factor gene *VP* 1 (*afVP* 1).

Gene Name:	Function:	Developmental Expression:
α-amylase (*AMY* 2/1) (*Avena fatua*) [1]	Starch hydrolysis	Germination Specific: Expression is repressed by *VP* 1 in maize.[5]
Em (wheat) [2]	?	Embryo Maturation: Expression is activated by *VP* 1 in maize.[6]
af 10 (*Avena fatua*) [3]	?	General.
afVP 1 (*Avena fatua*)	Embryo Transcription Factor[4]	Embryo Maturation in maize.[4]

Table 1: Function and expression patterns of genes used in this study. 1, Hooley *et al.* 1991, 2, Williamson *et al.* 1985, 3, Jones 1996, 4, McCarty *et al.* 1991, 5, Hoecker *et al.* 1995, 6, Vasil *et al.* 1995.

We were particularly interested in analysing the expression of the *A. fatua VP* 1 homologue under the conditions described. The *VP* 1 transcription factor has

previously been shown to control embryo maturation in maize, and recent evidence suggests that *ABI* 3 represses post-germination developmental processes during embryogenesis (Nambara *et al*. 1995). Our initial hypothesis was that the *A. fatua* homologue of *VP* 1 (*afVP* 1) may regulate processes involved with embryo dormancy following imbibition of the mature seed, by maintaining embryos in the dormant state and inhibiting the dormancy/germination transition. If this was true, then expression of the *A. fatua* homologue of *VP* 1 should be linked to the dormant phenotype in imbibed mature seed, and not be limited to embryogenesis. We cloned the *A. fatua* homologue of *VP* 1 (*afVP* 1, see section below) and used this cDNA to study expression characteristics of the corresponding RNA. Experiments analyzing the expression of other genes in this study used homologous probes (*AMY* 2/1, *af* 10) and a heterologous probe from wheat (*Em*). Expression of *Em* related RNAs was studied because this gene has been shown to be transcriptionally activated by *VP* 1 in maize during embryogenesis (McCarty *et al*. 1991, Vasil *et al*. 1995). Transcription of the α-amylase gene *AMY* 6-4 has been shown to be specifically repressed by VP 1 in developing barley seeds, and this gene is a classic marker for germination (Hoecker *et al*. 1995).

PolyA-containing RNA was extracted from seeds and analysed by northern blot using radioactively labelled DNA probes. Initially we investigated the expression of all the genes in freshly harvested seeds left to imbibe for 48 h (figure 2a). Differences in primary dormancy due to genotype were most pronounced at this time (figure 1a). *AMY*-related gene expression was correlated with lines showing germination (AN51 and CS40). The *af* 10 RNA was expressed in all inbred lines, regardless of phenotype (as were polyubiquitin-related RNAs, data not shown). Expression levels of *afEm* and *afVP* 1 RNAs were all increased in lines showing a dormant phenotype (AN51,

AN127, SH99, Bampton and Rewe). Next we investigated expression levels of these RNAs in selected lines that had been allowed to after-ripen (figure 2b). There was no difference in expression of all the different transcripts between fresh and after-ripened seed of the non dormant line CS40 (ND). There was an increase in expression of *AMY* expression in after-ripened AN51 and Bampton (although the increase was much less in Bampton), and a large decrease in expression of the *afVP* 1 and *afEm* RNA's in after-ripened imbibed seed compared to fresh dormant seed.

The influence of temperature of dry seed storage on gene expression was analysed using seeds from the dormant inbred line Bampton (figure 2c). Seed was stored for 1 year at either 24°C or 4°C, and then imbibed for 48 h before RNA extraction. Levels of *AMY*-related RNA were high in imbibed seeds that had been stored at 24°C and fully after-ripened, but not in imbibed seed stored at 4°C that were still dormant. Levels of RNA corresponding to the *afVP* 1 and *afEm* genes were higher in imbibed seeds that had been stored at 4°C and were still dormant.

We analysed the influence of induction of secondary dormancy on gene expression using the lines CS40 and Bampton (figure 2d). The inductive conditions had little effect on the final CS40 seed germination phenotype (figure 1c) or on the expression patterns of RNAs analysed in this inbred line (figure 2c). Embryos showed high levels of germination, and high levels of *AMY* RNA, but low levels of *afEm* and *afVP* 1 RNAs. The same environmental conditions induced secondary dormancy in Bampton seeds (figures 1c), and associated changes in gene expression. *AMY* RNA was detected at very low levels in treated seeds, whereas *afVP* 1 and *afEm* RNAs were present at high levels in treated embryos (D) compared to untreated (ND) embryos.

We analysed whether the depth of dormancy (ie. the length of after-ripening time required to break dormancy) shown by seeds from the inbred lines was correlated to the expression levels of *afVP* 1 in the dry seed. Poly(A)-containing RNA was extracted from dry seeds of lines CS40, AN51 (both show very short after-ripening times, figure 1), Bampton and Rewe (both have long after-ripening requirements), and expression of *afVP* 1 RNA was analysed by northern blot (figure 3). RNA corresponding to *afVP* 1 was expressed at similar high levels in the seeds of lines Bampton and Rewe, whereas expression of *afVP* 1 RNA was much lower in seeds from lines CS40 and AN51. Comparison of the after-ripening period and levels of *afVP* 1 expression (figure 3) demonstrated a positive correlation between the length of time required for after-ripening to occur and the level of expression of *afVP* 1 RNA in the dry seed.

Discussion

We have shown that genetic background and environment interact in the dry seed to control the subsequent developmental pathway, and gene expression programmes, of the embryo following imbibition. This study showed a strong correlation between the dormant phenotype and expression of *afVP 1* RNA in both the dry and imbibed seed. The results obtained suggest two new features of the biology of *VP* 1/*ABI* 3-related transcription factor family. Firstly, the results indicate that a switching mechanism in the dry seed results in differential expression of *afVP* 1 following imbibition. This mechanism results in increased expression of *afVP* 1 in dormant imbibed embryos from mature seeds, and reduced expression during the initiation of germination. Secondly, the results suggest a new function for *VP* 1 related transcription factors during dormancy in addition to already described functions in embryogenesis, as

regulators of post-imbibition dormancy-related processes.

The different inbred lines used in this study showed different degrees of primary dormancy and rates of after-ripening. In all cases, degree of dormancy and rate of after-ripening were positively correlated, indicating that the two processes were related. Temperature of storage also influenced after-ripening, a low temperature increasing the after-ripening period. This suggests that temperature effects the mechanism regulating after-ripening. Dormancy could be re-introduced (secondary dormancy) into embryos by a specific treatment, but only to embryos of those lines that originally showed primary dormancy. These results show that dormancy can only be re-introduced into embryos that have the capacity for primary dormancy, ie. those that have a genotype conferring dormancy. The results also suggest that primary and secondary embryo dormancy are both controlled by the same genetic loci responsible for the maintenance of primary dormancy. Those embryos showing primary dormancy also had the capacity for secondary dormancy. These results suggest that the switch mechanism operating in mature seeds may show some features of reversibility (figure 5). This model predicts that the switch determines the degree of primary dormancy, but can also be reactivated (reversed) by environmental conditions to induce secondary dormancy following loss of primary dormancy. In mature seeds, the switch controls the developmental decision of whether the seed will become dormant or germinate on imbibition, and of gene expression programmes in imbibed embryos (see below). A bistable switch has previously been postulated to control dormancy (Trewavas 1987) by interactions between kinases and phosphatases. The product of the *ABI* 1 gene from Arabidopsis (mutant *abi* 1 effects include disruption of dormancy) is a calcium-modulated phosphatase and could possibly fulfil this role (Leung *et al*. 1994).

Our analysis of gene expression following imbibition of seeds shows that dormant and germinating embryos carry out very different expression programmes. Other studies have shown that several genes of unknown function are up regulated in dormant embryos (Johnson *et al.* 1995, Li and Foley 1995). We have shown that expression of the *afVP* 1 gene was positively correlated to the dormant phenotype under all the conditions we tested, in both the dry and imbibed seed. In addition, *afEm* RNA showed a similar pattern of regulation. These results suggest therefore that these RNA's are regulated by developmental decisions that occur in the mature seed (figure 5). In particular it is noteworthy that *afVP* 1 expression in the dry seed was shown to be correlated with the depth of dormancy shown by inbred lines. That is, those lines that take longest to after-ripen contained the most *afVP* 1 RNA the dry seed, whereas those lines that after-ripen very quickly contained very low levels (figure 3). Thus the amount of *afVP* 1 RNA in the seed at the very onset of imbibition may determine to some degree the dormancy/germination fate of the seed, and this RNA is laid down in the seed during the final stages of embryo maturation.

The observation of positive correlation between *afVP* 1 expression in the dry seed and after-ripening requirement shows that this gene could be used as a molecular marker for dormancy potential/after-ripening time.

High levels of expression in the dry seed would indicate a higher degree of dormancy/ longer after-ripening requirement. The relationship between *afVP* 1 expression in dry and imbibed seed, and embryo genotype indicates that this gene may represent previously described *A. fatua* loci *L1* or *L2* (Jana *et al.* 1979, Jana *et al.* 1988), which influence the degree of dormancy.

Previous work has demonstrated that *VP 1/ABI 3* act in the maturation stage of embryogenesis (Hattori *et al.*, 1992, McCarty *et al.* 1991, Nambara *et al.* 1995, Parcy *et al.* 1994), and do not function following seed desiccation, (for example *ABI 3* RNA expression is reduced rapidly on imbibition of mature Arabidopsis seed [Parcy *et al.* 1994]). The up-regulation of the *afVP 1* RNA in dormant *A. fatua* embryos suggests that the encoded protein may play a role in maintaining the dormant state. Our results suggest that one function for afVP 1 in dormant embryos could be the transcriptional activation of the *A. fatua Em* gene. Maize *VP 1* has previously been shown to regulate *Em* during embryo maturation by activation of *Em* transcription through specific *cis*-elements (Vasil *et al.* 1995), and *afVP 1* could function in a similar way. It would be interesting to analyse the relationship between genes regulated by *afVP 1* in dormant embryos and during embryogenesis to define if this transcription factor functions in a similar or different way in these two different developmental states. Recent results show that *ABI 3* is also involved in repressing post-germination developmental processes during embryogenesis (Nambara *et al.* 1995). Another function of *afVP 1* in imbibed dormant seeds may therefore be the inhibition of germination-related processes (and germination-related gene expression such as α-amylase).

Example 2: Isolation of afVP1 - cDNA library construction and manipulation of nucleic acids

cDNA library construction was carried out as previously described (Holdsworth *et al.* 1992) using poly-A containing RNA from mature embryos of inbred line Bampton. Oligo dT primed cDNA was ligated into the vector λ-MOSS*lox* (Palazzolo *et al.* 1990), and screened according to the manufacturers recommendations (Amersham International plc, UK). Five-prime RACE (rapid

amplification of cDNA ends) was carried out using the Marathon cDNA amplification kit (Clontech Laboratories Inc, CA, USA). RACE-PCR was primed with a synthetic oligonucleotide corresponding to positions 878-898 of the full-length *afVP* 1 cDNA. Ligation and sub-cloning of DNA fragments were carried out as described in Sambrook *et al.* (1989). Sequencing of cloned RACE-PCR amplification products was performed manually by the dideoxy chain termination method (Sanger *et al.* 1977). Sequencing of λMOSS*lox* subclones was done using a DuPont Genesis 2000 Automated Sequencer (Univ. of Bristol Molecular Recognition Centre, UK). DNA sequence analysis was carried out using the MacVector™ and AssemblyLine™ programmes (Oxford Molecular Group plc, UK) and GCG8 (University of Wisconsin Genetics Computer Group version 8 [Genetics Computer Group 1994]).

Results

The DNA sequence corresponding to the *afVP* 1 RNA was obtained by a combination of cDNA cloning and 5'RACE (rapid amplification of cDNA ends). The combined length predicted from these sequences is 2338 bases for the full-length RNA, which is similar to the size observed in Northern Blot analysis of *afVP* 1.
The predicted protein is 662 amino acids long, smaller than all other VP 1/ ABI 3 homologues. The protein sequence of *afVP* 1 derived from the cDNA was compared to predicted protein sequences of homologues from maize (VP 1, McCarty *et al.* 1991), rice (osVP 1, Hattori *et al.* 1994), Arabidopsis (ABI 3, Giruadat *et al.* 1992) and *Phaseolus vulgaris* (ALF 1, Bob *et al.* 1995) using the GCG8 programme Pileup (Genetics Computer Group 1994).

Analysis of the predicted protein sequence of *afVP* 1 shows that it is highly similar to other VP 1/ABI 3-related proteins, particularly in the four regions

previously shown to be highly conserved. These regions may be involved in protein structure or be conserved functional domains. The region between amino acids 386 and 407, BR2 (Basic Region 2), in VP 1 has previously been shown to interact *in-vitro* with several different classes of transcription factor, including EmBP 1, previously shown to be involved in the regulation of the *Em* gene (Hill *et al*. 1996). The predicted protein sequence of afVP 1 shows high homology with VP 1/ABI 3 in this region, suggesting a similar functional role in *A. fatua*. The *in-vivo* importance of the BR2 region for the function of this transcription factor family is indicated by the observation that BR 2 occurs at position 439-475 in ABI 3, and the severe allele *abi* 3-4 contains a mutation that converts Gln 417 to a premature stop codon (Giraudat *et al*. 1992, figure 3). In addition, the fourth (and largest) highly conserved region lies downstream of the *abi* 3-4 premature stop codon, suggesting an important role for this region also, although no function has yet been proposed for this region. Other regions of the protein, including those shown in maize to regulate *Em* transcription and *AMY* repression show low homology, and may be responsible for different functions of the proteins or differences in efficiency of interaction with other proteins.

EXAMPLE 3 - Cloning taVP 1 using afVP 1

Plant material;

Wheat variety Soleil was obtained from Dr John Flintham (John Innes Centre, Norwich, UK). This variety was chosen because it has a high resistance to PHS. Mature seeds were obtained from plants grown outdoors during the summer. Air dry seeds were stored in the dark at -20°C to maintain dormancy levels prior to RNA extractions.

Germination assays

These were conducted as described for afVP1.

Extraction and analysis of RNA from wheat seeds

Poly-A containing RNA was extracted from seeds as described previously (Rushton et al 1995). Otherwise the process was as for afVP1.

cDNA library construction, screening and manipulation of nucleic acids

cDNA library construction was carried out a previously described (Holdsworth et al 1992) using poly-A containing RNA from mature embryos of the wheat variety Soleil. 500 Soleil seeds (harvested 1996) were imbibed at 20°C for 8 hours. Embryos were dissected out and poly(A)RNA extracted (approximately 65μg). Northern analysis using a fragment of afVP 1 (bases 600-1892) as a probe confirmed the presence of wheat homologues in the RNA preparation. 5μg poly(A)RNA was used to construct the cDNA library (Amersham plc cDNA synthesis kit). Oligo dT primed cDNA was ligated into the vector λXAP II (Stratagene). 10^6 plaque-forming units from the primary library were amplified to obtain an amplified library with final titre of 10^7 pfu/ml. This library was screened using the hybridisation and washing conditions described above for Northern analysis. Wheat VP 1 homologues were identified using as a probe either a fragment of the afVP 1 cDNA from the 5' end (basis 1-892 of afVP 1), or a fragment from the middle of afVP 1 (bases 600-1892). A total of 8 cDNA clones were purified and characterised, all showing specific hybridisation of the afVP 1 cDNA. Sequencing of cloned cDNA products was performed manually by the dideoxy chain termination method (Sanger et al 1997), or for the longest cDNA clone (Clone 10, 2.3kbp)

via contracting out (Oswel, University of Southampton). DNA sequence analysis was carried out using the MacVector™ and AssemblyLine™ programmes (Oxford Molecular Group plc, UK) and GCG8 (University of Wisconsin Genetics Computer Group version 8 [Genetics Computer Group 1994]). The wheat cDNA clones shared 81% DNA sequence identity with the afVP 1 clone.

EXAMPLE 4 - Method for Reducing PHS in Wheat by use of afVP1 sequence

Wheat transformation is conveniently carried out according to Barcelo & Lazzeri (1995) in Methods in Molecular Biology, Vol 49. Chapter 9, pp 113-124; Ed H Jones, Humana Press, Totowa, New Jersey. This employs microprojectile bombardment of immature inflorescence and scutellum tissues; the content of this paper is indicative of the ability of those skilled in the art to perform wheat transformation without burden. Other common methods for transforming wheat are discussed by Christou in Trends in Plant Science (1996)1, 12: 423-431, and by Chang et al in US patent No. 5,610,042. For the avoidance of doubt any content of these documents not forming part of the common general knowledge is herein incorporated by reference. Briefly, the full-length afVP 1 cDNA (or a truncated derivative) is cloned into a wheat transformation vector downstream of the rice actin promoter, which confers constitutive expression of afVP 1, or the ubiquitin promoter. Immature embryos or inflorescences are bombarded with gold microcarriers coated with plasmid DNA. Explants are cultured, selected and plants are regenerated. Transgene expression is then assayed e.g. using a GUS marker.

More specifically transgenic wheat containing sense and antisense afVP1 constructs was produced and analysed as follows:

a. Description of afVP1 constructions:

Sense and antisense versions of afVP1 were cloned into the plant transformation/expression vector pUPLN, that contains the ubiquitin promoter upstream of a multiple cloning region (MCR). This plant transformation vector was constructed in the laboratory of Dr. Paul Lazzeri (IACR-Rothamsted). It contains the Ubiquitin promoter and first intron and exon, and the NOS terminator DNA sequence from the plamid pAHC17 (Christensen and Quail, Transgenic Research 5, 213-218; 1996). These DNA sequences were introduced into the plamid pSP72 to create pUPLN.

A NotI DNA fragment containing only the complete afVP1 cDNA was introduced into pUPLN at the Not I site within the MCR. Subclone's were identified as containing the afVP 1 in the "sense" of "antisense" orientation with respect to the ubiquitin promoter, by digestion of plamid DNA with SpeI (that distinguishes afVP 1 insert orientation because both the afVP 1 cDNA and pUPLN contain a single site each).

b. Transformation of wheat

This was carried out as described above. pUPLN plamid DNA containing afVP 1 in either sense of antisense orientation were separately transformed into wheat (cultivars used were; Cadenza, Canon, Riband, Imp and Avans, information is shown for Cadenza and Canon).

c. Identification of transgenic plants:

i) Using PCR to show wheat plants contain afVP 1 DNA sequence:

PCR was carried out as already described.

Oligonucleotide's specific to afVP 1 were used with wheat genomic DNA derived from putative transgenic plants to amplify a portion of the afVP 1 cDNA within the pUPLN plamid integrated into wheat genomic DNA.

The oligonucleotides were:

(5': 63967: bases 1410-1429 of EMBL deposited afVP 1 sequence):

5' CAA CTC ATG GTC CCG AAT CC 3'

(5': afVP1EPRIME: bases 2285-2302 of EMBL deposited afVP 1 sequence):

5' GCT TGT TAG ACG AAT TGA C 3'

The results of this experiment demonstrated that transgenic plants have been identified that separately contain copies of sense and antisense afVP 1 cDNA.

ii) Northern analysis of transgenic antisense plants:

Total RNA was extracted from leaf material using a Qiagen™ kit.

N-blot production and analysis carried out as described in Sambrook *et al* (1989, supra). The probe used was a radiolabelled an afVP 1, 1.4 Kbp Not/Hind III DNA fragment, containing 1.4kbp at the 3 prime end of the cDNA.

The results of this experiment are shown in Fig 9. This

shows that the transgenic plants analysed express antisense afVP 1 in leaf material to a very high level. One specific hybridising transcript is detected in leaf total RNA from transgenic line #33 (within cultivar Cadenza). Control RNA derived from untransformed leaf material of the same cultivar did not show hybridisation to the afVP 1 probe.

EXAMPLE 5 - Mapping taVP1 and OsVP1

Mapping in wheat

An af*VP1* cDNA clone ('lars10' - taVP1) was used to identify the chromosomal location of *taVp1* loci in hexaploid wheat and identify a restriction fragment length polymorphism (RFLP) which was used for genetic mapping. Using nullisomic-tetrasomic lines of the wheat cultivar 'Chinese Spring' in genomic Southern analysis, the presence of *taVp1* homologs on each group 3 chromosome was established . Two RFLPs were identified between the wheat cultivars 'Chinese Spring' and 'Synthetic' in an *Eco*R1 DNA digest. This enabled *taVp1* to be mapped on chromosomes 3A and 3D using the F_2 population from 'ChineseSpring' × 'Synthetic' cross of Devos *et al* (1992).

The 3B orthologue was not polymorphic in digests with *Eco*R1, *Eco*RV, *Dra*I or *Hind*III and was not mapped. Mapping data were incorporated into the existing linkage map for this cross using MAPMAKER version 3.0. On chromosome 3A, *Xlars10 (taVp1)* is positioned distal to marker *Xpsr549* by 12.1 cM and proximal to marker Xabg389 by 3.9 cM. The 3D homoeolocus is distal by 12.1 cM to *Xpsr 170* and proximal to *Xpsr* 1067 by 5.5 cM. These two gene locations are consistent with the location shown in Figure 7, the the interval *Xpsr549 - Xwg110* on the wheat

group 3 consensus map of Gale et al. (1995), about 30 cM from the centromere. *Xlars10 (taVp1)* shows clear recombination with *R* loci which map about 60 cM from the centromere. Thus, although *Xlars10 (taVp1)* and *R* are linked, they are clearly distinct genes.

Thus the results show that both wheat carries a *Vp1* orthologue in loose genetic linkage with previously mapped genes controlling seedcoat pigments (*R* loci in wheat). It is proposed that this genetic separation between the two loci reflects separate roles for *taVp1* and *R* respectively in zygotic and maternal dormancy mechanisms. To date no QTL mapping the proximity of *Xlars10* had been detected in wheat (Anderson et al 1993). QTL for malting quality that map to the long arm of chromosome 3 in barley vary in position between genetic crosses and different environments, and it is not clear whether these QTL reflect effects on dormancy (compare for example, Hayes et al. 1993 c.f Oberthur et al. 1995).

Assigment of VP1 cDNA clones to the A and D genomes of wheat

Background

An alignment of sequences from six *Vp1* cDNA clones demonstrated that the clones fall into two main groups characterised by single base pair differences at a number of places along the sequence, illustrated in Fig 11 for a section of cDNA (group 1: clones 4, 5 and 10; group 2: clones 3, 6 and 9). Each group appears to represent a single genomic copy gene (one set from a gene on a chromosome 3A, the other on 3D). The differences between the clones on each family may therefore have arisen by post-transcriptional modifications (e.g. differential splicing to form mature mRNA). This suggests that there may be advantages in tagging taVP1 mRNA and/or

polypeptides (rather than genomic alleles) to assess taVP1 function (and hence PHS or other dormancy traits).

Methods

PCR primers were designed to specifically amplify each sequence group from genomic DNA. Fig. 11 illustrates the location of the 3' end of the forward primers, designed to coincide with the base pair differences existing between each group:

primer 4 forward: 5' AATATCTGATACGCGGCGTGAAGGTG3'

primer 9 forward: 5' AGGATCTAGCCAAGCACAAGAATGG3'

The 3' end of the reverse primers were designed in a similar way:

primer 4 reverse: 5'GCCCATATGAACTCGATCGATTGAC 3'

primer 9 reverse: 5'GTTGTCCATATGAACTCGATCGATTC 3'

The reaction conditions are described below:

1X Taq buffer (Boehringer)

1.2 mM $MgCl_2$

200 μM of dGTP, dATP, dUTP and dCTP

0.4 μM of each primer

1.5 U/100 μl Taq polymerase (Boehringer)

50 ng genomic DNA

The total reaction volume was 50 μl. The PCR cycles were:

Five cycles of:

94°C 1 minute

70°C 1 minute

72°C 1 minute

Thirty cycles of:

94°C 45 seconds

65°C 1 minute

72°C 1 minute

Results

The result of the PCR on various templates is shown in Fig. 12. Plasmid DNA from clones four (4) and nine (9) represent the two sequence groups and act as control

templates; primer set 4 is specific to one group and primer set nine is specific to the other. For genomic DNA from a nullisomic-tetrasomic line of Chinese Spring in which the 3A chromosome is absent (N3A/T3D), amplification does not occur. However, when the 3B chromosome (in N3B/T3A lines) or 3D chromosome (in N3D/T3A lines) is absent amplification can still occur. This demonstrates that clone 4 represents the gene located on chromosome 3A.

For the primer set designed to the other sequence group, amplification occurs using DNA from nulli-tetra lines in which the 3A and 3B chromosomes are missing but amplification is abolished in lines missing 3D. This demonstrates that clone 9 represents the gene located on chromosome 3D.

Genomic clones for all three genome copies have been identified in a Soleil genomic library. Genome-specific primers will be useful for targetting each copy of *Vp1* and studying correlations of particular *Vp1* alleles with dormancy/preharvest sprouting. They may also have particular utility in assessing introgression into specific wheat genomes, for instance from wild relatives.

EXAMPLE 6 - Use of the taVP1 as a marker for dormancy in breeding programmes

Superior alleles of *taVp1* may be identified by cloning (e.g. using probes/primers based on the sequences disclosed herein e.g. in Fig 10) and sequencing alleles from a variety of lines, and correlating the sequence with the PHS properties of those plants. PCR primers which are specific for "superior" alleles can then be used to select preferred genotypes. This allows early-generation selection against PHS using a rapid, small-scale method, in contrast with current practise of

delaying selection until late generations and using extremely cumbersome and rather unreliable empirical dormancy tests.

Dormancy may be improved by selection for high levels of expression of fully-functional alleles, firstly by selecting parents carrying desirable genomic copies of *taVp1* gene(s), then by selecting progeny which express these alleles strongly at appropriate stages of seed development and maturation.

Desirable alleles may be obtained from within the existent wheat (*Triticum aestivum*) genepool, but may also be detected and transferred from one or more of the many wild or cultivated relatives of wheat, for which established methods are available for the introduction of "alien" variation into the hexaploid. The recombining of wheat/alien chromosomes is a standard technique, see e.g. M.D.Gale & T.E.Miller.1987. The introduction of alien genetic variation into wheat. pp173-210 in: Wheat Breeding Its Scientific Basis. Ed. F.G.H.Lupton. Chapman & Hall. Wheats carrying such alleles could then be crossed into a commercial breeding programme and varieties resistant to PHS due to the presence of superior alleles could be identified eg. by PCR using allele-specific primers.

For applications in which dormancy is undesirable, eg. grain for brewing, the same information and techniques could be employed to select in the reverse direction, i.e. to fix defective or poorly expressed copies of *taVp1* (malting wheat).

EXAMPLE 7 - Use of the taVP1 for predicting the susceptibility to PHS

A taVP1 based test for predicting the onset of PHS in

commercial crops would enable farmers to prioritise harvesting of high-quality varieties. Present attempts to do this only use weather data (P.S.Kettlewell, G.D.Lunn, B.J.Major, R.K.Scott, P.Gate & F.Couvreur,1995. A possible scheme for pre-harvest prediction of Hagberg Falling Number and sprouting of wheat in the U.K. and France. p35-41 in: Pre-Harvest Sprouting in Cereals 1995. Eds. K.Noda & D.J.Mares. Centre for Academic Societies Japan). The level of effective *taVp1* activity in the seed may be measured e.g. using a PCR-based or antibody assays in order to predict susceptibility to PHS.

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CLAIMS

1 An isolated nucleic acid molecule encoding afVP1 comprising a nucleotide sequence identical to Seq ID No 1 or degeneratively equivalent thereto.

2 An isolated nucleic acid comprising a homologous variant of the nucleotide sequence of claim 1 having at least about 70% sequence identity therewith.

3 A nucleic acid as claimed in claim 2 which is a derivative by way of one or more of addition, insertion, deletion or substitution of one or more nucleotides in the nucleic acid and which encodes a polypeptide having afVP1 activity.

4 A nucleic acid as claimed in claim 3 wherein the derivative is altered with respect to its transcriptional activation domain or repressor domain.

5 A nucleic acid as claimed in claim 2 which is a mutant or allelic variant of Seq ID No 1 obtainable from *Avena fatua*

6 A nucleic acid which is complementary to the nucleic acid of any one of claims 1 to 5

7 A nucleic acid molecule for use as a probe or primer, said molecule having a contiguous sequence of at least 18, 21 or 24 nucleotides which sequence is shown in Seq ID No 1 and is characteristic of Seq ID No 1, or the complement thereof.

8 A nucleic acid as claimed in claim 7 wherein the contiguous sequence is at least 150 nucleotides.

9 A nucleic acid as claimed in claim 7 wherein the

contiguous seuqence is a probe which corresponds to bases 1-892, or 600 to 892 of Seq ID No 1, or a primer which corresponds to bases 1398-1417 or 2272-2290 of Seq ID No 1.

10 A method of identifying and/or cloning a nucleic acid molecule which is homologous variant of the nucleotide sequence of claim 1, which method employs the probe or primer as claimed in any one of claims 7 to 9.

11 A method as claimed in claim 10 wherein the plant species is not *A fatua*.

12 A method as claimed in claim 10 or claim 11 comprising the steps of:
(a) providing a preparation of nucleic acid from a plant cell,
(b) providing a nucleic acid molecule as claimed in any one of claims 7 to 9,
(c) contacting nucleic acid in said preparation with said nucleic acid molecule under conditions for hybridisation of said nucleic acid molecule to any said gene or homologue in said preparation, and
(d) identifying said gene or homologue if present by its hybridisation with said nucleic acid molecule.

13. A method as claimed in claim 10 or claim 11 comprising the steps of:
(a) providing a preparation of nucleic acid from a plant cell,
(b) providing a pair of nucleic acid molecule primers suitable for PCR as claimed in any one claims 7 to 9,
(c) contacting nucleic acid in said preparation with said primers under conditions for performance of PCR,
(d) performing PCR and determining the presence or absence of an amplified PCR product.

14 An isolated nucleic acid molecule which is:
(a) a homologous variant of the nucleic acid of claim 1, and
(b) which is obtainable from a wheat variety by the method of any one of claims 10 to 13.

15 A nucleic acid as claimed in claim 14 which comprises a nucleotide sequence identical to any one of the nucleotide sequences designated: taVP1 (Seq ID No 2); clone 2; clone 3; clone 4; clone 5; clone 6; clone 9 in Figure 10.

16 An isolated nucleic acid comprising a homologous variant of the nucleotide sequence of claim 15 having about at least 70% sequence identity therewith.

17 A nucleic acid which is complementary to the nucleic acid of any one of claims 14 to 16

18 A recombinant vector comprising the nucleic acid of any one of claims 1 to 6 or claims 14 to 17.

19 A vector as claimed in claim 18 which is capable of replicating in a suitable host.

20 A vector as claimed in claim 18 or claim 19 wherein the nucleic acid is operably linked to a promoter or other regulatory element for transcription in a host cell.

21 A vector as claimed in claim 20 further comprising any one or more of the following: a terminator sequence; a polyadenylation sequence; an enhancer sequence; a marker gene.

22 A vector as claimed in claim 20 or claim 21 wherein the promoter is an inducible promoter.

23 A vector as claimed in claim 22 wherein the promoter is developmentally regulated.

24 A vector as claimed in any one of claims 19 to 23 which is a plant vector.

25 A vector as claimed in claim 24 comprising a selectable genetic marker which confers a selectable phenotype selected from: resistance to antibiotics or herbicides.

26 A method comprising the step of introducing a vector as claimed in any one of claims 19 to 25 into a cell.

27 A method for transforming a plant cell, comprising a method as claimed in claim 26, and further comprising the step of causing or allowing recombination between the vector and the plant cell genome to introduce the nucleic acid into the genome.

28 A host cell comprising a vector as claimed in any one of claims 19 to 25.

29 A host cell transformed with a vector as claimed in any one of claims 19 to 25.

30 A host cell as claimed in claim 28 or claim 29 which is a plant cell.

31 A host cell as claimed in claim 30 which is in a plant.

32 A method for producing a transgenic plant comprising a method as claimed in claim 27 and further comprising the step of regenerating a plant from the transformed cell.

33 A polypeptide encoded by the nucleic acid of any one of claims 1 to 5 or claims 14 to 16.

34 A method of producing a polypeptide comprising the step of causing or allowing the expression from a nucleic acid of any one of claims 1 to 5 or claims 14 to 16 in a suitable host cell.

35 An antibody or fragment thereof, or a polypeptide comprising the antigen-binding domain of the antibody, capable of specifically binding the polypeptide of claim 33.

36 A method of producing the antibody or fragment as claimed in claim 35 comprising the step of immunising a mammal with a polypeptide according to claim 33.

37 A method of identifying and/or isolating a polypeptide having one or more afVP1 or taVP1 epitopes, said method comprising use of a polypeptide comprising the antigen-binding domain of the antibody of claim 35.

38. A method for influencing the dormancy characteristics of a seed or grain, the method comprising use of any one or more of the following: all or part of the nucleic acid of any one of claims 1 to 6 or claims 14 to 17; the polypeptide of claim 33; the antibody or fragment or polypeptide comprising the antigen-binding site thereof of claim 35.

39 A method as claimed in claim 38 wherein the dormancy characteristic is either the viviparous or PHS phenotype.

40 A method as claimed in claim 38 or claim 39 comprising the step of causing or allowing expression of a nucleic acid according to any one of claims 1 to 6 or claims 14 to 17 within the seed or grain.

41 A method as claimed in any one of claims 38 to 40 comprising suppressing the dormancy characteristic in the seed or grain.

42 A method as claimed in claim 41 comprising any one of the following: causing or allowing the transcription of part of the nucleic acid of any one of claims 1 to 5 or claims 14 to 16 in the cell such as to co-suppress the expression of an endogenous gene; causing or allowing the transcription of nucleic acid of claim 6 or claim 17 in the cell; causing or allowing the expression of a polypeptide comprising the antigen-binding domain of the antibody of claim 35 in the cell.

43 A plant comprising the cell of any one of claims 28 to 31.

44 A plant as claimed in claim 43 produced by the method of claim 32.

45 A plant which is the progeny of a plant as claimed in claim 43 or claim 44.

46 A plant as claimed in any one of claims 43 to 45 which is wheat.

47 A method for assessing the PHS and/or other dormancy related properties of a wheat plant, the method comprising use of the molecular marker taVP1 which occurs in the interval between loci *Xwg110* and *Xpsr549* on the wheat group 3 consensus map.

48 A method as claimed in claim 47 wherein the individual taVP1 alleles present in the wheat are assessed on the basis of nucleic acid sequence and/or protein sequence.

49 A method as claimed in claim 48 wherein the result of the nucleic acid sequence and/or protein sequence assessment is directly correlated with the PHS and/or other dormancy related properties of a wheat by comparison with a further taVP1 nucleic acid sequence and/or protein sequence.

50 A method as claimed in any one of claims 47 to 49 comprising use of any one or more of the following: a taVP1 allele and/or genome specific probes; one or more allele and/or genome specific primers for PCR; restriction analysis; assessment of quality or quantity of taVP1 mRNA or protein product; allele specific amplification; RFLP analysis; AFLP analysis; RT-PCR; antibody analysis.

51 A method as claimed in claim 50 comprising use of two primers to amplify a taVP1 allele, at least one of the primers having a nucleotide sequence of at least 15 nucleotides, which sequence is shown in, or complementary to, all or part of a nucleic acid as claimed in claim 15.

52. A method as claimed in claim 51 comprising the steps of:
(a) providing a preparation of nucleic acid from a plant cell,
(b) providing a pair of nucleic acid molecule primers suitable for PCR, at least one of the primers having a nucleotide sequence of at least 15 nucleotides, which sequence is shown in, or complementary to, all or part of a nucleic acid as claimed in claim 15,
(c) contacting nucleic acid in said preparation with said primers under conditions for performance of PCR,
(d) performing PCR and determining the presence or absence of an amplified PCR product.

53 A nucleic acid molecule for use as a probe or

primer, said molecule having a contiguous sequence of at least 18, 21 or 24 nucleotides which sequence is shown in a nucleic acid as claimed in claim 15, or the complement thereof.

54 A nucleic acid molecule for use as a probe or primer, said molecule having a contiguous sequence of at least 18, 21 or 24 nucleotides which sequence is shown in two or more of the nucleic acids of claim 15, or the complement thereof.

55 A nucleic acid as claimed in claim 53 or claim 54 which is capable of distinguising individual taVP1 alleles

56 A nucleic acid as claimed in claim 55 selected from any of the following:

CAT CTC AGG TGT GGA GCA TGC

CGG CAC ATC TCA GAT TTT GGC CC

GCG GCA GCA GGG TGC GAG G

GCG GCA GCA GGT GCA TGC ATG

57 A nucleic acid as claimed in claim 53 or claim 54 which is capable of distinguising genomic taVP1 loci.

58 A nucleic acid as claimed in claim 57 selected from any of the following:

(primer 4 forward): 5' AATATCTGATACGCGGCGTGAAGGTG 3'
(primer 9 forward): 5' AGGATCTAGCCAAGCACAAGAATGG 3'
(primer 4 reverse): 5' GCCCATATGAACTCGATCGATTGAC 3'
(primer 9 reverse): 5' GTTGTCCATATGAACTCGATCGATTC 3'

59 A composition comprising a pair of primers capable of distinguishing individual taVP1 alleles and/or genomic loci, at least one of said primers being a nucleic acid as claimed in any one of claims 53 to 58.

60 A composition as claimed in claim 59 wherein the primer pair comprises a non-specific primer and an allele and/or genome specific primer.

61 A kit for assessing the PHS and/or other dormancy related properties of a wheat, the kit comprising the nucleic acid molecule of any one of claims 53 to 58, or the composition of claim 59 or claim 60.

62 A method for generating a wheat plant having a desired characteristic as regards PHS and/or other dormancy related traits comprising the steps of:
(a) selecting a parent plant having desired PHS and/or other dormancy related properties,
(b) crossing that line with a second parental plant, and
(c) assessing the progeny by use of a method as claimed in any of claims 47 to 52,
(d) selecting progeny on the basis of the assessment in step(c).

63 A method as claimed in claim 62 wherein the taVP1 alleles present in the progeny are assessed using DNA from the seed tissue prior to sowing.

64 A method as claimed in claim 62 or claim 63 wherein the selection of the parent line(s) and/or progeny is done using the method of claim 49.

65 A method as claimed in any one of claims 62 to 64 wherein the parent plant having desired PHS and/or other dormancy related properties is obtained from: an existing variety genepool; prepared mutants from within an elite

genepool; a cultivated wheat; a wild related species of the plant.

66 A method as claimed in any one of claims 62 to 65 wherein functional taVP1 alleles which are expressed strongly at appropriate stages of seed development and maturation are selected in the parent and/or progeny.

67 A method as claimed in any one of claims 62 to 65 wherein defective or poorly expressed taVP1 alleles are selected in the parent and/or progeny

68 A method of producing a wheat cultivar comprising the method of any one of claims 62 to 67.

69 A plant as claimed in claim 46, or a method as claimed in any one of claims 47 to 52 or claim 62 to 68 wherein the wheat is a white or amber grained wheat.

70 A method as claimed in any one of claims 62 to 68 wherein the parent and/or progeny are additionally selected on the basis of red grain colour

71 A method of producing seed by cultivating the cultivar of producing by the method of claim 68 and harvesting seed therefrom.

72 A part or propagule of the plant of any one of claims 43 to 46, or as produced by the method of any one of claims 62 to 68.

73 A part or propagule as claimed in claim 72 which is a seed or grain.

74 A commodity comprising, or obtainable from, the seed or grain of claim 73.

75 A commodity as claimed in claim 74 comprising milled or malted grain or flour.

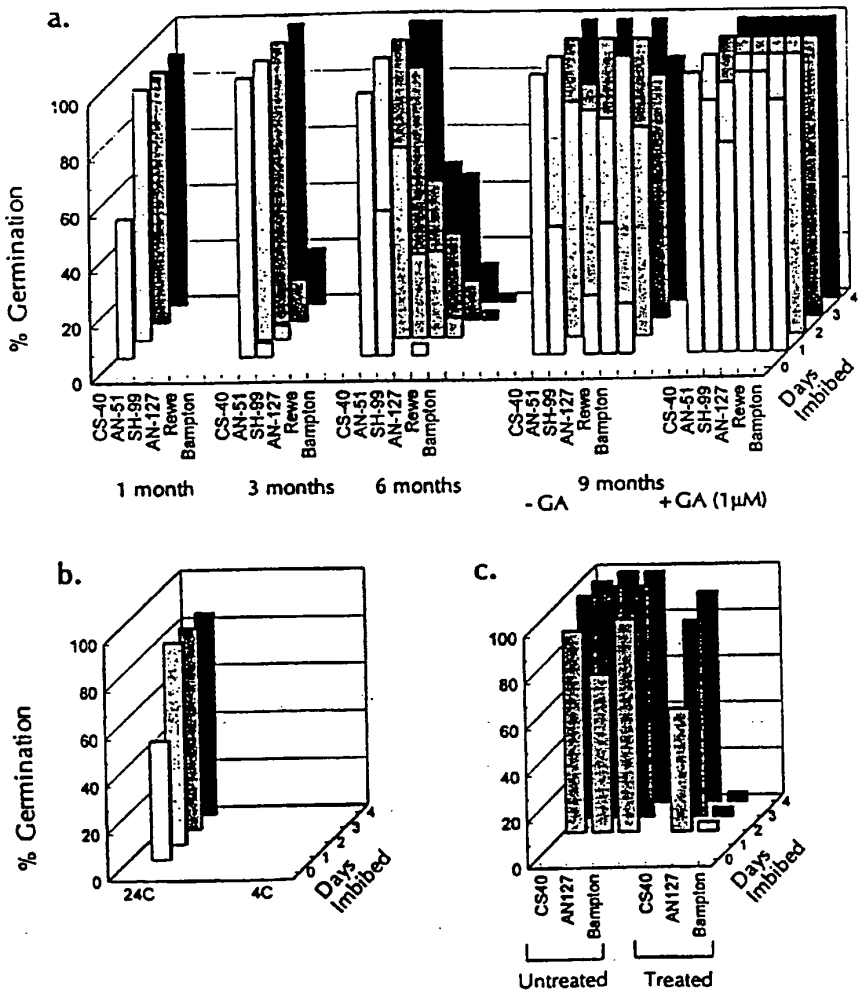

a.
% Germination
100
80
60
40
20
0
CS-40
AN-51
SH-99
AN-127
Rewe
Bampton
Days Imbibed
0
1
2
3
4
1 month
3 months
6 months
9 months
-GA
+GA (1μM)
b.
% Germination
24C
4C
Days Imbibed
c.
CS40
AN127
Bampton
Untreated
Treated
Days Imbibed

FIG.1

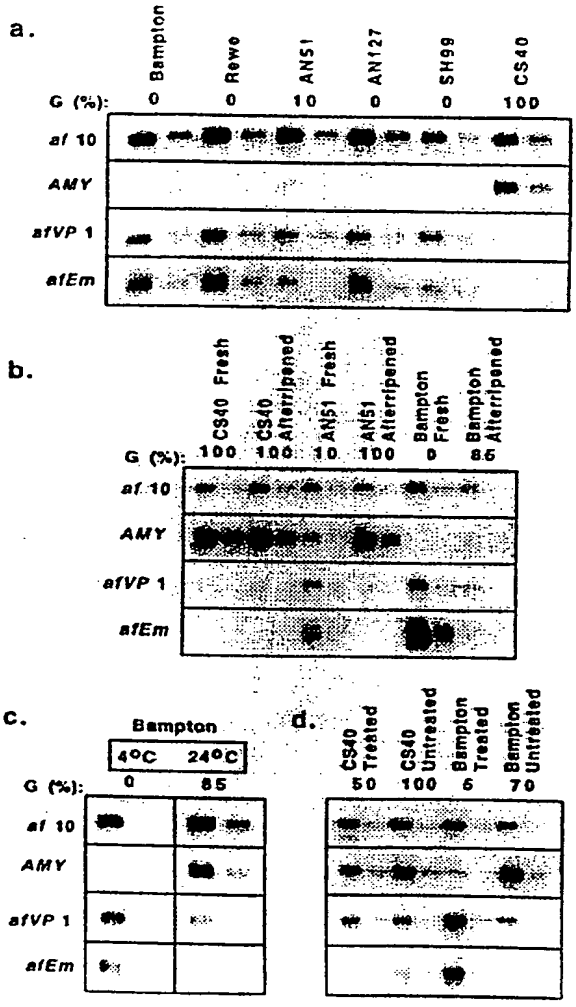
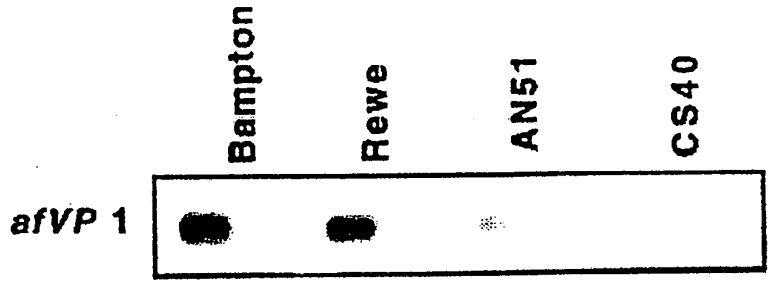

a.
Bampton
Rewe
AN51
AN127
SH99
CS40
G (%):
0
0
10
0
0
100
af 10
AMY
afVP 1
afEm
b.
CS40 Fresh
CS40 Afterripened
AN51 Fresh
AN51 Afterripened
Bampton Fresh
Bampton Afterripened
G (%):
100
100
10
100
0
85
af 10
AMY
afVP 1
afEm
c.
Bampton
4°C
24°C
G (%):
0
85
af 10
AMY
afVP 1
afEm
d.
CS40 Treated
CS40 Untreated
Bampton Treated
Bampton Untreated
50
100
6
70

FIG. 2



G (%)	Bampton	Rewe	AN51	CS40
1month	0	0	0	90
3 months	0	0	25	100
6 months	10	14	100	100

FIG. 3

```
   1 GCACACCCCT TCTTCCCTCC TTCCCTCCCT CCCTCCTCCT GCCTTCCCTT TGCATGGACG
  61 CCTCCGCCGG CTCCTCGCCG CCGCCGCACT CGCAGGAGAA CCCGCCCAAG CACGGTGGAG
 121 GCCGCGGGAA GCGTGCGGGG GAGATCCGGA AGGGAGAGGC GGCCACGGCG GATGACTTTA
 181 TGTTCGCGGA AGATACCTTC CCGTCCCTCC CGGATTTCCC TTGCCTCTCC TCCCGTTCAA
 241 GCTCCACCTT CTCCTCCTCA TCCTCCTCCA ACTCATCCAG CACCCACGCC GCCGCGGGAC
 301 GCGGCGTGGC CGTTGTCGCG GACGCCCGAA GGCGCCTCGG GGAGCCCTCC GATCCTGCTG
 361 CCGCGGGGGA CGATGACGTG CTCGACGACA TCGACGAGCT GCTCAACTCT GCCACGCTCT
 421 CCGACTCCAT GCCCTGGGAG GACGAGCCGC TCTTCCCCGA CGACGTTGGC ATGATGATAG
 481 AGGACGCCAT CTCCCACCAG CCGCCCGCCA CGGGCCACCG CGGAGCCAGG AACGCTGCAT
 541 CATCGGAGGC GGCTGCTGGT GGTGGTGGAC AGGATTCCTC GTCGGCGGAC GACCTGCCGC
 601 GGTTCTTCAT GGAGTGGCTG ACGAACAACC GCGACTGCAT CTCCGCCGAG GACCTCCGCA
 661 GCATCCGCCT CCGCCGCTCG ACCATCGAGG CCGCGGCGGC GCGGCTCGGT GGAGGGCGGC
 721 AGGGCACCAT GCAGCTGCTC AAGCTCATCC TCACATGGGT GCAGAACCAC CATCTGCAGA
 781 AGAAGCGCGC CCGCGTCGAC GACGAGCTCC CCAGCCCCGG CGCAAACCCG GGTTACGAGT
 841 TCCCCGCGGA GACAGTTGCC CCCGCCACAT CCTGGCTCAT GCCCTACCAA CAAGCTTATG
 901 GAAGAGAGGC GATCTACCCG AACGCCGCCG CCACCGGGCA GTACCCATTC CAGCAGGGCG
 961 GCAGCACGAG CAGCGTGGTG GTGAGCAGCC AGCCGTTCTC CCCGCCGGCG CCGGTGGCCG
1021 ACATGCAGGC GGCGAACATG CCCTGGCCGC AGCAGTACGC GGCGTTCCCC GGCGCTGCGC
1081 CATACCCGAT GCCGCCGCCG CAGCCGTTGG CGGCGGCCGG ATTCGGCGTG TGCCCGCAGC
1141 CCTTGGCCGG GGTGAAGCCG TCGGCGAGCA AGGAGGCCCG GAAGAAGCGT ATGGCGAGGC
1201 AGCGCCGCCT CTCCTGCCTG CAGCATCAGC GGAGCCAGCA GCTGAATCTG GGCCAGATCC
1261 AGAACGCCAT GATCCATCCG CAGCAGGAGG TGCCGTTCTC TCCCCGCTCC GCGCACTCGG
1321 TGCCTGTCTC ACCGCCGTCG CCCGGCGGCT GGTGCGGGCT CTGGCCGCCG CCCTCCGTCC
1381 AAGTCCAGGG CCAGGGCCAA CTCATGGTCC CGAATCCGCT GTCGACAAAG CCCAGTTCCT
1441 CCTCGAGGCA GAAGGCGCAG AAACCCTCGC CGGACGCAGG AGCAAGACCG CCGTCGTCCG
1501 GCGCGCAGCA GGGTGCGAAG CCGGGGGCGG ACAAGAATCT GCGGTTTCTG CTGCAGAAGG
1561 TGCTGAAGCA GAGCGACGTC GGCGCCCTCG GCCGCATCGT GCTCCCCAAA GAAGCGGAGA
1621 CGCACCTGCC GGAGCTCAAG ACGAGGGACG GCATCTCCAT CCCCATGGAG GACATCGGCA
1681 CCTCTCGGGT CTGGAGCATG CGGTACCGGT TTTGGCCTAA CAACAAGAGC AGAATGTATC
1741 TCCTTGAGAA CACTGGGGAC TTTGTTCGCT CAAACGAGCT GCAGGAGGGC GACTTCATCG
1801 TGATTTACTC AGATGTCAAG TCGGGCAAAT ATCTGATACG TGGTGTGAAG GTAAGACCGC
1861 CGCAGGATCT AGCGAAGCAG AAGCATGGCA GTCTAGAGAA AGGCAGCACC TCAGATGCGA
1921 TGCCCTGCGC TGAAGACGGT GGCGCCGAGG CAGGCGGCTG CAAGGGGAAG TCTCCGCACG
1981 GCGTTAGGCG GTCTCGCCAG GAGGCTGCGT CCATGAACCA GATGGCGGTG AGCATCTGAA
2041 AGAACAGCCC TAGACGATCC ACCATTGAAG ACTTAGCTAG CTCGTGTATA CATGATGTTG
2101 ATGATCAAAT CGATCTCTGG CACCGTTGTA TTATCCGTAG TACTCTAGCC CTAGGGATGG
2161 TTATATATTA AAGTAGCTAT CAGTCCGATG TGACGACTAA AGAATGCATG GTTTGGTTCG
2221 TTAAAACCCT GTAACCCTGT ACATGCATGA ACATAATAAC TTATTTGTCG TGTCAATTCG
2281 TCTAACAAGC AGACTAGTTC CTGCCGTAAA AAAAAAAAAA AAAA
```

FIG. 4(a)

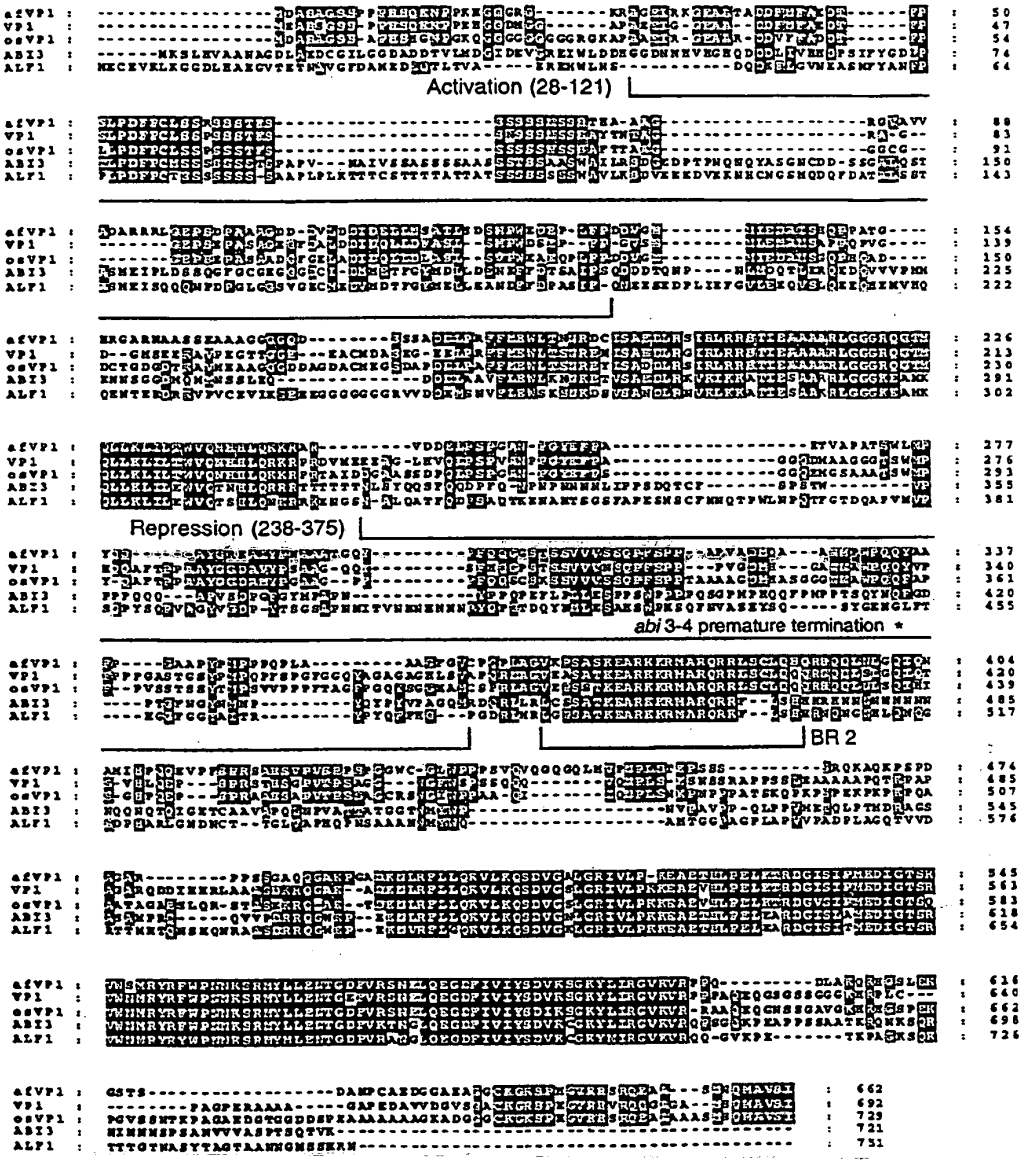

Activation (28-121)
Repression (238-375)
abi 3-4 premature termination *
BR 2

FIG. 4(b)

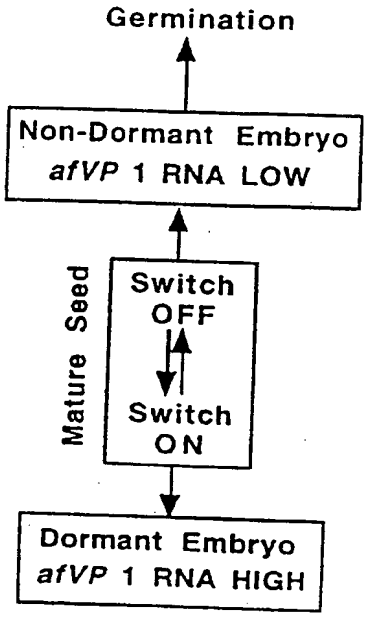

Germination
Non-Dormant Embryo
afVP 1 RNA LOW
Mature Seed
Switch
OFF
Switch
ON
Dormant Embryo
afVP 1 RNA HIGH

FIG. 5

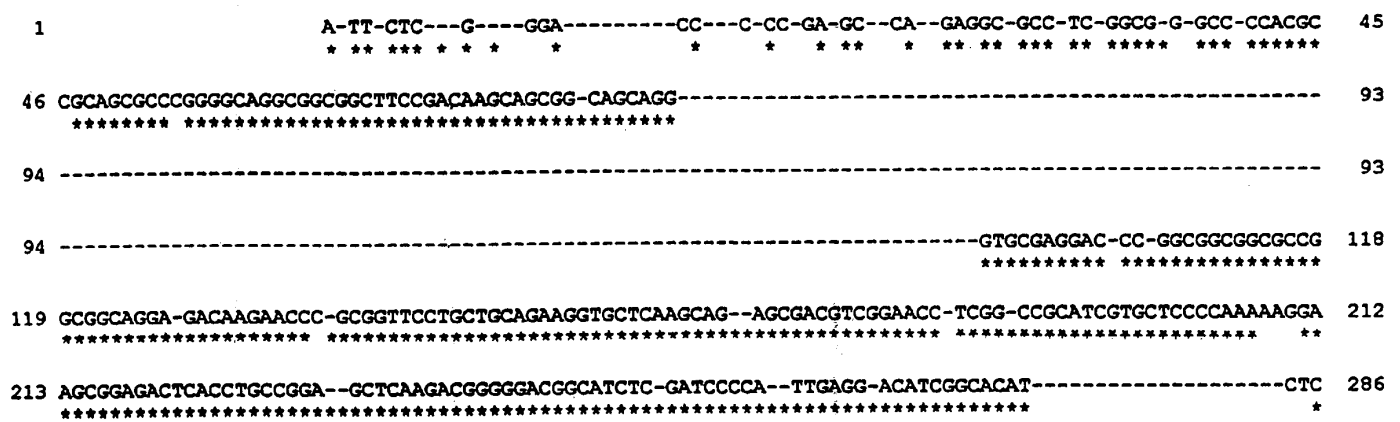


Fig. 6A

```
287 AGATTTTGGCCCAACACAAGA-GCAGAATGTA--TCTTCTAGAGAA-CACTGGTGACTTTGTTCGG-TCGAAT-AGCTGCAGGAGG-GTGATTTCATCG 379
    ************************************** ** * ************ ********************** ************************

380 TGCTTTAC--TCTGATGTCAAGTC-GGCAAATATCT--AT-C-C-GGCGTGAAGGTGAGAGCGCA-ACAGGATCTAGCCAAGCACAA--AATGCCAGT-- 466
    ********************************** *********** *** * ********************* *********** * ** ******

467 --CCAGAGAAAGGCGGGGGCTTCC----TGAA-G-CGGGCGGAGAAGAC--GGCGGCTGC-AGGAGAAGCCCCCCCACGGCGTCCGGCGATCTCGCCAGGA 555
    ***************** ***** ***    *** **  ****  * ** * ** *** * ******  * ** ***** ************************

556 GGCCGCCTCCATGAACCAGATGGC-GGTGAGCATC-TGAAATGAGCA-GCTCGCC--GTCCGATCCACCATTGAA-GA-TCAGT-TAGCT-AGCTCAAGT 646
    ************************** ************************ ****  *   ********** *********      * ********************

647 ATACCC-TTGA-TGATGATCAAAT--CGATC-TCTCGTT-TA-GATCCGTGCTTC-G-GT-A-TGCTGTAGCCCTA-GTTAGGGATGATGATACTAAAGT 734
    *****  ********************** ** *** **** ** ************ * **** ********************************** ***********

735 A-CTA-TCGGTCAGATGTGAC-CT-AA-AATGCATGGTCCGTGCTGTT-AACC-GTAT-AA-GCTGTAACCCTT-T-T---AAAAAAAAAAAAAA 817
```

Fig. 6B

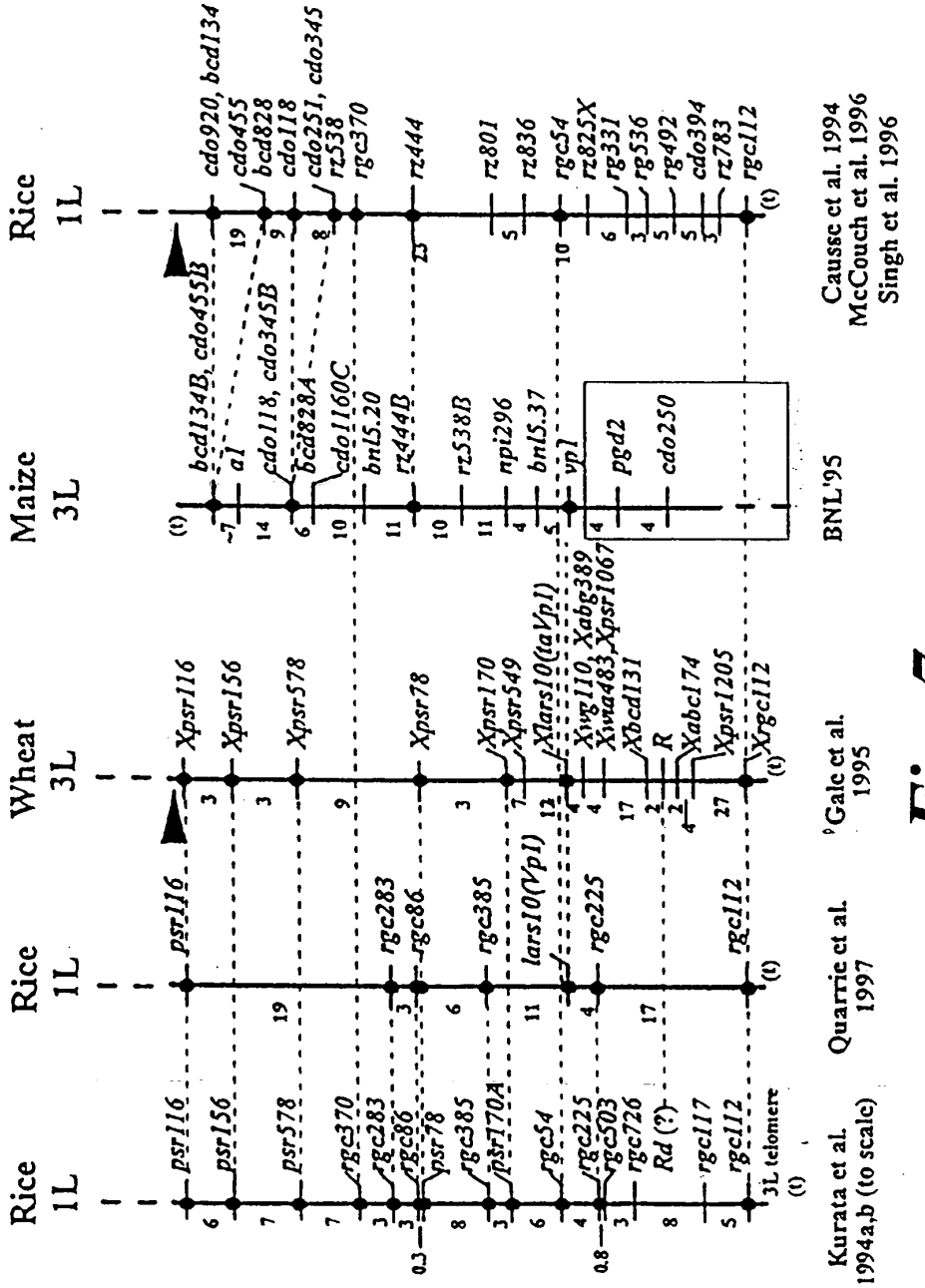

Rice 1L
Rice 1L
Wheat 3L
Maize 3L
Rice 1L
Kurata et al. 1994a,b (to scale)
Quarrie et al. 1997
Gale et al. 1995
BNL'95
Causse et al. 1994
McCouch et al. 1996
Singh et al. 1996
psr116
psr156
psr578
rgc370
rgc283
rgc86
psr78
rgc385
psr170A
rgc54
rgc225
rgc503
rgc726
Rd (?)
rgc117
rgc112
3L telomere
rgc112
lars10(Vp1)
rgc225
rgc283
rgc86
rgc385
Xpsr116
Xpsr156
Xpsr578
Xpsr78
Xpsr170
Xpsr549
Xlars10(laVp1)
Xwg110, Xabg389
Xwa483, Xpsr1067
Xbcd131
R
Xabc174
Xpsr1205
Xrgc112
bcd134B, cdo455B
a1
cdo118, cdo345B
bcd828A
cdo1160C
bnl5.20
rz444B
rz538B
npi296
bnl5.37
vp1
pgd2
cdo250
cdo920, bcd134
cdo455
bcd828
cdo118
cdo251, cdo345
rz538
rgc370
rz444
rz801
rz836
rgc54
rz825X
rg331
rg536
rg492
cdo394
rz783
rgc112

Fig. 7

```
clone 5 seq        1
clone 6 seq        1
clone 9 seq 7/8    1                                                                                                    0
tavpl (clone 10)   1 GGCACGAGGACGACTTCATGTTCGCGCACGATACCTTCCCGGCCCTCCCGGACTTCCCTTGCCTCTCCTCGCCGTCGAGCTCCACCTTCTCCTCCTCGTC  100

clone 5 seq        1
clone 6 seq        1
clone 9 seq 7/8    1                                                                                                    0
tavpl            101 GTCTTCCAACTCCTCCAGCGCCTTCACCCGCGCCGTGGGGGCAGGCGGGCGCGGGGGCGAGAGTGCGCGCGGCGAGCCGTCCGAGCCTGCCGCGGCCGGG  200

clone 5 seq        1
clone 6 seq        1
clone 9 seq 7/8    1                                                                                                    0
tavpl            201 GACGGGATGGACGACCTCTCCGACATCGACCACCTGCTCGACTTCGCATCCATCAACGAGGACGTCCCTTGGGACGACGAGCCGCTCTTCCCCGACGTCG  300

clone 5 seq        1
clone 6 seq        1
clone 9 seq 7/8    1                                                                                                    0
tavpl            301 GGATGATGCTGGAGGACGTCATCTCCGAGCAGCAGCAGTTGCAACCTCCGGCGGGCCACGGCACGGCCGGGAGAACGGCGTCGCATGCGGCTGCTGGTGG  400

clone 5 seq        1
clone 6 seq        1
clone 9 seq 7/8    1                                                                                                    0
tavpl            401 AGGAGAGGATGCCTTCATGGGTGGCGGCGGCACGGGGAGCGCGGCGGACGACCTGCCGCGCTTCTTCATGGAGTGGCTCAAGAACAACCGCGACTGCATC  500
```

Fig. 8A

```
clone 5 seq          1                                                                                                  0
clone 6 seq          1                                                                                                  0
clone 9 seq 7/8      1                                        GGCACGAGCCACCATCGAGGCCGCGGCCGCGCGCCTCGGTGGGGGGCGCCAGGGCACCATGCAGCTGCTCA   71
tavpl              501 TCGGCCGAGGACCTCCGCAGCATCCGCCTCCGTCGATCCACCATCGAGGCCGCGGCCGCGCGCCTCGGTGGGGGGCGCCAGGGCACCATGCAGCTGCTCA  600

clone 5 seq          1                                                                                                  0
clone 6 seq          1                                                                                                  0
clone 9 seq 7/8     72 AGCTCATCCTCACCTGGGTGCAGAACCACCACCTGCAGAAGAAGCGCCCCCCGCGTCGGCGCCATGGATCAGGAGGCGCCGCCGGCAGGAGGCCAGCTCCC  171
tavpl              601 AGCTCATCCTCACCTGGGTGCAGAACCACCACCTGCAGAAGAAGCGCCCCCGCGTCGGCGCCATGGATCAGGAGGCGCTGCCGGCAGGAGGCCAGCTCCC  700

clone 5 seq          1                                                                                                  0
clone 6 seq          1                                                                                                  0
clone 9 seq 7/8    172 CAGCCCCGGCGCAAACCCCGGCTACGAATTCCCCGCGGAGACGGGTGCCGCCGCTAACACATCTTGGATGCCCTACCAGGCCTTCTCGCCAACTGGATCC  271
tavpl              701 TAGCCCCGGCGCAAACCCCGGCTACGAATTCCCCGCGGAGACGGGTGCCGCCGCTGCCACATCTTGGATTCCCTACCAGGCCTTCTCGCCAACTGGATCC  800

clone 5 seq          1                                                                                                  0
clone 6 seq          1                                                                                                  0
clone 9 seq 7/8    272 TACGGCGGCGAGGCGATCTACCCGTTCCAGCAGGGCTGCAGCACGAGCAGCGTGGCCGTGAGCAGCCAGCCGTTCTCCCCGCCGGCGGCGCCCGACATGC  371
tavpl              801 TACGGCGGCGAGGCGATCTACCCGTTCCAGCAGGGCTGCAGCACGAGCAGCGTGGGCGTGAGCAGCCAGCCGTTCTCCCCGCCGGCGGCGCCCGACATGC  900

clone 5 seq          1                                                                                                  0
clone 6 seq          1                                                                                                  0
clone 9 seq 7/8    372 ACGCCGGGGCCTGGCCGCTTCAGTACGCGGCGTTCGTCCCAGCTGGGGCCACATCCGCAGGCACTCAAACATACCCGATGCCGCCGCCGGGGGCCGTGCC  471
tavpl              901 ACGCCGGGGCCTGGCCGCTGCAGTACGCGGCGTTCGTCCCAGCTGGGGCCACATCCGCAGGCACTCAAACATACCCGATGCCGCCGCCGGGGGCCGTGCC 1000

clone 5 seq          1                                                                                GGCACGAGGCGG   12
clone 6 seq          1                                                    CGCAGCGGATGGAACCGGCGGCGACCAGGGAGGCCCGGAAGAAGAGGATGGCGAGGCAGCGG   62
clone 9 seq 7/8    472 GCAGCCGTTCGCGGCCCCCGGATTCGCCGGGCAGTTCCCGCAGCGGATGGAACCGGCGGCGACCAGGGAGGCCCGGAAGAAGAGGATGGCGAGGCAGCGG  571
tavpl             1001 GCAGCCGTTCGCGGCCCCCGGATTCGCCGGGCAGTTCCCGCAGCGGATGGAGCCGGCGGCGACCAGGGAGGCCCGGAAGAAGAGGATGGCGAGGCAGCGG 1100
                                                                                                  *  * *  ****

clone 5 seq         13 CGCCTGTCGTGCCTGCAGCAGCAGCGGAGCCAGCAGCTGAATCTGAGCCAGATCCAAACCGGCGGCTTCCCTCAAGAGCCATCCCCCCGCGCGGCGCACT  112
clone 6 seq         63 CGCCTGTCGTGCCTGCAGCAGCAGCGGAGCCAGCAGCTGAATCTGAGCCAGATCCAAAGCGGCGGCTTCCCTCAAGAACCATCCCCCCGCGCGGCGCACT  162
clone 9 seq 7/8    572 CGCCTGTCGTGCCTGCAGCAGCAGCGGAGCCAGCAGCTGAATCTGAGCCAGATCCAAAGCGGCGGCTTCCCTCAAGAACCATCCCCCCGCGCGGCGCACT  671
tavpl             1101 CGCCTGTCGTGCCTGCAGCAGCAGCGGAGCCAGCAGCTGAATCTGAGCCAGATCCAAACCGGCGGCTTCCCTCAAGAGCCATCCCCCCGCGCGGCGCACT 1200
                       ************************************************************ ****************** ***********************

clone 5 seq        113 CGGCGCCGGTCACTCCGCCGTCGTCTGGCTGGGGAGGCCTCTGGACGCAACAAGCCGTCCAGAGCCAGCCCCATGGCCAGCTCATGGTCCAGGTCCCGAA  212
clone 6 seq        163 CGGCGCCGGTCACGCCGCCCTCTTCCGGCTGGGGAGGCCTCTGGTCGCAGCATGCCGTCCAGGGCCAGCCCCATGGCCAGCTCATGGTCCAGGTTCCGAA  262
clone 9 seq 7/8    672 CGGCGCCGGTCACGCCGCCCTCTTCCGGCTGGGGAGGCCTCTGGTCGCAGCATGCCGTCCAGGGCCAGCCCCATGGCCAGCTCATGGTCCAGGTTCCGAA  771
tavpl             1201 CGGCGCCGGTCACGCCGCCGTCGTCTGGCTGGGGAGGCCTCTGGACGCAACAAGCCGTCCAGAGCCAGCCCCATGGCCAGCTCATGGTCCAGGTCCCGAA 1300
                       ************** ****** ** ** ******************** **** ** ********** ********************************** *****
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Fig. 8B

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Clone 5 seq      213 TCCGCTGTCGACGAAGTCCAATTCCTCAAGGCAGAAGCAGCAAAAACCCTCGCCGGACGCAGCAGCGAGGCCGCCCTCCGGCGGCGCCGCCACGCCGCAG  312
Clone 6 seq      263 TCCGCTGTCGACGAAGTCCAATTCCTCGAGGCAGAAGCAGCAAAAACCCTCGCCGGATGCAGCAGCGAGGCCGCCCTCCGGCGGCGCCGCCACGCAGCAG  362
Clone 9 seq 7/8  772 TCCGCTGTCGACGAAGTCCAATTCCTCGAGGCAGAAGCAGCAAAAACCCTCGCCGGATGCAGCAGCGAGGCCGCCCTCCGGCGGCGCCGCCACGCAGCAG  870
tavp1           1301 TCCGCTGTCGACGAAGTCCAATTCCTCAAGGCAGAAGCAGCAAAAACCCTCGCCGGACGCAGCAGCGAGGCCGCCCTCCGGCGGCGCCGCCACGCCGCAG 1400
                     **************************** **************************** ************************ * ********** ****

Clone 5 seq      313 CGCCCGGGGCAGGCGGCGGCTTCCGACAAGCAGCGGCAGCAGG---------------------------------------------------------  355
Clone 6 seq      363 CGCCCGGGGCAGGCGGCGGCTTCCGACAAGCAGCGGCAGCAGG---------------------------------------------------------  405
Clone 9 seq 7/8  871 CGCCCGGGGCAGGCGGCGGCTTCCGACAAGCAGCGGCAGCAGGTGCATGCATGCACGAACACCTCTTGCCATCCATCCATCGATCGCCATCCCGCATAGA  970
tavp1           1401 CGCCCGGGGCAGGCGGCGGCTTCCGACAAGCAGCGGCAGCAGGTGCATGCATGCACGAACACCTCTTGCCATCCATCCATCGATCGCCATCCCGCATAGA 1500
                     ********************************************

Clone 5 seq      356 ---------------------------------------------------------------------------------------------------  355
Clone 6 seq      406 ---------------------------------------------------------------------------------------------------  405
Clone 9 seq 7/8  971 ATCACAAGCCATTGCTCCCCAAATAAGTGTGCGTACATCGTAAGAGACGCACATCGCTGTCCAGCGATAGGATATCCCCGCATCGCCATCCCGCATAGAA 1070
tavp1           1501 ATCACAAGCCATTGCTCCCCAAATAAGTG---------------------------------------------------------------------- 1529

Clone 5 seq      356 ---------------------------------------------------------GTGCGAGGACGCCGGCGGCGGCGCCGGCGGCAG  388
Clone 6 seq      406 ---------------------------------------------------------GTGCGAGGACGCCGGCGGCGGCGCCGGCGGCAG  438
Clone 9 seq 7/8 1071 TCACAAGCCATTGCTCCCCTGCACGGTGAATTGCGTTTCTCAACGAGGTTCCGTGCATGCGCGCAGGGTGCGAGGACGCCGGCGGCGGCGCCGGCGGCAG 1170
tavp1           1530 ---------------------------------------------------------GTGCGAGGACGCCGGCGGCGGCGCCGGCGGCAG 1562
                                                                                 ********************************

Clone 5 seq      389 GAGACAAGAACCCGCGGTTCCTGCTGCAGAAGGTGCTCAAGCAGAGCGACGTCGGAACCCTCGGCCGCATCGTGCTCCCCAAA---GAAGCGGAGACTCA  485
Clone 6 seq      439 GAGACAAGAACCTGCGGTTCCTGCTGCAGAAGGTGCTCAAGCAGAGCGACGTCGGAACCCTCGGCCGCATCGTGCTCCCCAAA---GAAGCGGAGACTCA  535
Clone 9 seq 7/8 1171 GAGACAAGAACCTGCGGTTCCTGCTGCAGAAGGTGCTCAAGCAGAGCGACGTCGGAACCCTCGGCCGCATCGTGCTCCCCAAA---GAAGCGGAGACTCA  535
tavp1           1563 GAGACAAGAACCCGCGGTTCCTGCTGCAGAAGGTGCTCAAGCAGAGCGACGTCGGAACCCTCGGCCGCATCGTGCTCCCCAAAAAGGAAGCGGAGACTCA 1270
                     ************ ********************************************************************************   ***************

Clone 5 seq      486 CCTGCCGGAGCTCAAGACGGGGGACGGCATCTCGATCCCCATTGAGGACATCGGCACATCTCAG--------------------ATTTTGGCCCAACAAC  565
Clone 6 seq      536 CCTGCCGGAGCTCAAGACGGGGGACGGCATCTCGATCCCCATTGAGGACATCGGCACATCTCAGGTGTGGAGCATGCGGTACCGATTTTGGCCCAACAAC  635
Clone 9 seq 7/8 1271 CCTGCCGGAGCTCAAGACGGGGGACGGCATCTCGATCCCCATTGAGGACATCGGCACATCTCAGGTGTGGAGCATGCGGTACCGATTTTGGCCCAACAAC 1370
tavp1           1660 CCTGCCGGAGCTCAAGACGGGGGACGGCATCTCGATCCCCATTGAGGACATCGGCACATCTCAG--------------------ATTTTGGCCCAACAAC 1739
                     ********************************************************************   ****************
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Fig. 8C

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Clone 5 seq       566 AAGAGCAGAATGTATCTTC-TAGAGAACACTGGTGACTTTGTTCGGTCGAATGAGCTGCAGGAGGGTGATTTCATCGTGCTTTACTCTGATGTCAAGTCG  664
Clone 6 seq       636 AAGAGCAGAATGTATCTTTCTGGAGAACACTGGAGACTTTGTTCGGTCGAATGAGCTGCAGGAGGGTGATTTCATCGTGCTTTACTCTGATGTCAAGTCG  735
Clone 9 seq 7/8  1371 AAGAGCAGAATGTATGTTG-TGGAGAACACTGGAGACTTTGTTCGGTCGAATGAGCTGCAGGAGGGTGATTTCATCGTGCTTTACTCTGATGTCAAGTCG 1469
tavp1            1740 AAGAGCAGAATGTATCTTC-TAGAGAACACTGGTGACTTTGTTCGGTCGAATGAGCTGCAGGAGGGTGATTTCATCGTGCTTTACTCTGATGTCAAGTCG 1838
                      *************** **  * *********** ********************************************************** 

Clone 5 seq       665 GGCAAATATCTGATACGCGGCGTGAAGGTGAGAGCGCAACAGGATCTAGCCAAGCACAAGAATGCCAGTCCAGAGAAAGGCGGGGCGTCCGACGTGAAGG  764
Clone 6 seq       736 GGCAAATATCTGATACGCGGCGTGAAGGTAAGAGCGCAACAGGATCTAGCCAAGCACAAGAATGGCAGTCCAGAGAAAGGTGGGGCGTCCGACGCGAAGG  835
Clone 9 seq 7/8  1470 GGCAAATATCTGATACGCGGCGTGAAGGTAAGAGCGCAACAGGATCTAGCCAAGCACAAGAATGGCAGTCCAGAGAAAGGTGGGGCGTCCGACGCGAAGG 1569
tavp1            1839 GGCAAATATCTGATACGCGGCGTGAAGGTGAGAGCGCAACAGGATCTAGCCAAGCACAAGAATGCCAGTCCAGAGAAAGGCGGGGCGTCCGACGTGAAGG 1938
                      ******************************** ************************************ *************** ************* ***** 

Clone 5 seq       765 CGGGCGGAGAAGACGGCGGCTGCAAGGAGAAGCCCCCCCCACTGCGTCCGGCGATCTCGCCAGGAGGCCGCCTCCATGAACCAGATGGCGGTGAGCATCTG  864
Clone 6 seq       836 CGGGCGCAGAAGACGGTGGTTGCAAAGAGAAGTCTCCGCACGGTGTCCGGCGATCTGGCCAGGAGGCCGCCTCCATGAACCAGATGGCCGTGAGCATCTG  935
Clone 9 seq 7/8  1570 CGGGCGCAGAAGACGGTGGTTGCAAAGAGAAGTCTCCGCACGGTGTCCGGCGATCTGGCCAGGAGGCCGCCTCCATGAACCAGATGGCCGTGAGCATCTG 1669
tavp1            1939 CGGGCGGAGAAGACGGCGGCTGCAAGGAGAAGCCCCCCCCACGGCGTCCGGCGATCTGCCAGGAGGCCGCCTCCATGAACCAGATGGCGGTGAGCATCTG 2038
                      ******* ********** ** ****** ****** * ** *** * ****************************************** ************ 

Clone 5 seq       865 AAATGAGCAGGCTCGC--CGTCCGATCCAC-ATTGAAGACTCAGTTAGCTAGCTGAAGTATACCCGTTGATGATGATCAAATCGATCTCTCGTTCTATGA  961
Clone 6 seq       936 AAATGAGCAGGCTCGCGCGGTCCGATCCCCCATTGAAGACT-ACTTAGCTAGCTGAAGTATACCTGTTGATGATGATCAAATCGATCTCCCGTTCTATGA 1034
Clone 9 seq 7/8  1670 AAATGAGCAGGCTCGCGCGGTCCGATCCCCCATTGAAGACT-ACTTAGCTAGCTGAAGTATACCTGTTGATGATGATCAAATCGATCTCCCGTTCTATGA 1768
tavp1            2039 AAATGAGCAGGCTCGC--CGTCCGATCCACCATTGAAGACTCAGTTAGCTAGCTGAAGTATACCCGTTGATGATGATCAAATCGATCTCTCGTTCTATGA 2136
                      *****************   ********* * ********** * ******************** ************************ ********** 

Clone 5 seq       962 TCCGTGCTTCCGTGTACTGCTGTAGCCCTAGTTAGGGATGATGATACTAAAGTAGCTATCGGTCAGATGTGACGCTGAAGAATGCATGGTCCGTGCTGTT 1061
Clone 6 seq      1035 TCCGTGCTTCCGTGTACTGCTGTAGCCCTAGTTAGGGATGGTGATACTAAAGTAGCTATCGGTCAGATGTGACGCTGAAGAATGCATGGTCCGTGCTGTT 1134
Clone 9 seq 7/8  1769 TCCGTGCTTCCGTGTACTGCTGTAGCCCTAGTTAGGGATGGTGATACTAAAGTAGCTATCGGTCAGATGTGACGCTGAAGAATGCATGGTCCGTGCTGTT 1868
tavp1            2137 TCCGTGCTTCCGTGTACTGCTGTAGCCCTAGTTAGGGATGATGATACTAAAGTAGCTATCGGTCAGATGTGACGCTGAAGAATGCATGGTCCGTGCTGTT 2236
                      ****************************************** ********************************************************** 

Clone 5 seq      1062 AAACCTGTATAAAGGCTGTAACCCTTCTGTACATGCATGAACATACCCTTA----------------------AAAAAAAAAAAAAAAAAAAAA  1133
Clone 6 seq      1135 AAACCTGTATAAAAG-TGTAA-CCTTCTGTTAAAAAAAAAAAAAAAAAAAAA                                              1185
Clone 9 seq 7/8  1869 AAACCTGTATAAAGGCTGTAACCCTTCTGTAAAAAAAAAAAAAAAAAA                                                    1916
tavp1            2237 AAACCTGTATAAAGGCTGTAACCCTTCTGTACATGCATGAACATACCCTTATTTGTTGTGTGTTGTCCTCCTAAAAAAAAAAAAAAAAAAAAAAAA 2332
                      ************** * ***** ********  *   *  ** * *
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Fig. 8D

Northern analysis of *afVP* 1 RNA expression in Wheat Leaf Tissue from Transformed and Untransformed (control) plants:

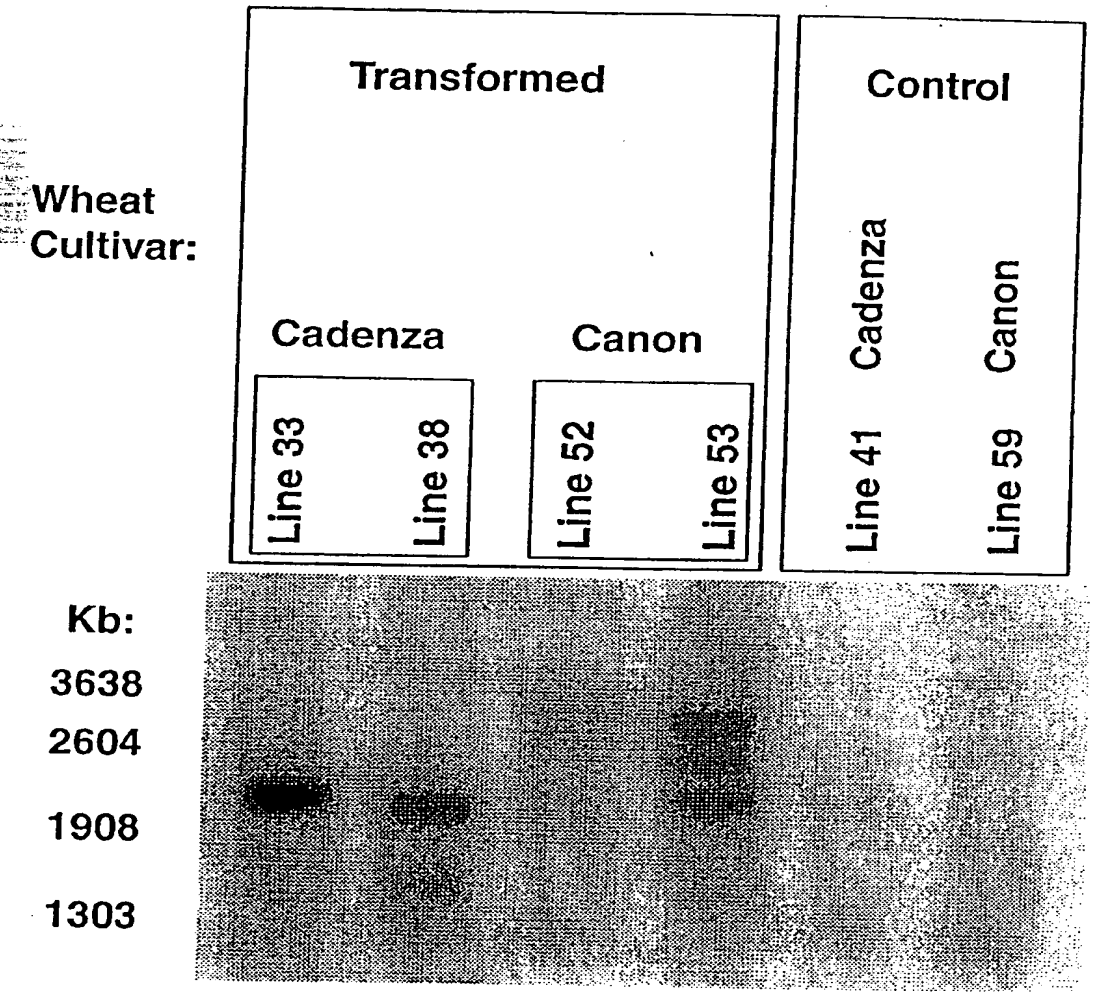


Fig. 9

GGCACGAGGACGACTTCATGTTCGCGCACGATACCTTCCCGGCCCTCCCGGACTTCCCTTGCCT
CTCCTCGCCGTCGAGCTCCACCTTCTCCTCCTCGTCGTCTTCCAACTCCTCCAGCGCCTTCACC
CGCGCCGTGGGGGCAGGCGGGCGCGGGGGCGAGAGTGCGCGCGGCGAGCCGTCCGAGCCTGCCG
CGGCCGGGGACGGGATGGACGACCTCTCCGACATCGACCACCTGCTCGACTTCGCATCCATCAA
CGAGGACGTCCCTTGGGACGACGAGCCGCTCTTCCCCGACGTCGGGATGATGCTGGAGGACGTC
ATCTCCGAGCAGCAGCAGTTGCAACCTCCGGCGGGCCACGGCACGGCCGGGAGAACGGCGTCGC
ATGCGGCTGCTGGTGGAGGAGAGGATGCCTTCATGGGTGGCGGCGGCACGGGGAGCGCGGCGGA
CGACCTGCCGCGCTTCTTCATGGAGTGGCTCAAGAACAACCGCGACTGCATCTCGGCCGAGGAC
CTCCGCAGCATCCGCCTCCGTCGATCCACCATCGAGGCCGCGGCCGCGCGCCTCGGTGGGGGGC
GCCAGGGCACCATGCAGCTGCTCAAGCTCATCCTCACCTGGGTGCAGAACCACCACCTGCAGAA
GAAGCGCCCCCGCGTCGGCGCCATGGATCAGGAGGCGCTGCCGGCAGGAGGCCAGCTCCCTAGC
CCCGGCGCAAACCCCGGCTACGAATTCCCCGCGGAGACGGGTGCCGCCGCTGCCACATCTTGGA
TTCCCTACCAGGCCTTCTCGCCAACTGGATCCTACGGCGGCGAGGCGATCTACCCGTTCCAGCA
GGGCTGCAGCACGAGCAGCGTGGGCGTGAGCAGCCAGCCGTTCTCCCCGCCGGCGGCGCCCGAC
ATGCACGCCGGGGCCTGGCCGCTGCAGTACGCGGCGTTCGTCCCAGCTGGGGCCACATCCGCAG
GCACTCAAACATACCCGATGCCGCCGCCGGGGGCCGTGCCGCAGCCGTTCGCGGCCCCCGGATT
CGCCGGGCAGTTCCCGCAGCGGATGGAGCCGGCGGCGACCAGGGAGGCCCGGAAGAAGAGGATG
GCGAGGCAGCGGCGCCTGTCGTGCCTGCAGCAGCAGCGGAGCCAGCAGCTGAATCTGAGCCAGA
TCCAAACCGGCGGCTTCCCTCAAGAGCCATCCCCCCGCGCGGCGCACTCGGCGCCGGTCACGCC
GCCGTCGTCTGGCTGGGGAGGCCTCTGGACGCAACAAGCCGTCCAGAGCCAGCCCCATGGCCAG
CTCATGGTCCAGGTCCCGAATCCGCTGTCGACGAAGTCCAATTCCTCAAGGCAGAAGCAGCAAA
AACCCTCGCCGGACGCAGCAGCGAGGCCGCCCTCCGGCGGCGCCGCCACGCCGCAGCGCCCGGG
GCAGGCGGCGGCTTCCGACAAGCAGCGGCAGCAGGTGCATGCATGCACGAACACCTCTTGCCAT
CCATCCATCGATCGCCATCCCGCATAGAATCACAAGCCATTGCTCCCCAAATAAGTGGTGCGAG
GACGCCGGCGGCGGCGCCGGCGGCAGGAGACAAGAACCCGCGGTTCCTGCTGCAGAAGGTGCTC
AAGCAGAGCGACGTCGGAACCCTCGGCCGCATCGTGCTCCCCAAAGAAGCGGAGACTCACCTGC
CGGAGCTCAAGACGGGGGACGGCATCTCGATCCCCATTGAGGACATCGGCACATCTCAGATTTT
GGCCCAACAACAAGAGCAGAATGTATCTTCTAGAGAACACTGGTGACTTTGTTCGGTCGAATGA
GCTGCAGGAGGGTGATTTCATCGTGCTTTACTCTGATGTCAAGTCGGGCAAATATCTGATACGC
GGCGTGAAGGTGAGAGCGCAACAGGATCTAGCCAAGCACAAGAATGCCAGTCCAGAGAAAGGCG
GGGCGTCCGACGTGAAGGCGGGCGGAGAAGACGGCGGCTGCAAGGAGAAGCCCCCCCACGGCGT
CCGGCGATCTCGCCAGGAGGCCGCCTCCATGAACCAGATGGCGGTGAGCATCTGAAATGAGCAG
GCTCGCCGTCCGATCCACCATTGAAGACTCAGTTAGCTAGCTCAAGTATACCCGTTGATGATGA
TCAAATCGATCTCTCGTTCTATGATCCGTGCTTCCGTGTACTGCTGTAGCCCTAGTTAGGGATG
ATGATACTAAAGTAGCTATCGGTCAGATGTGACGCTGAAGAATGCATGGTCCGTGCTGTTAAAC
CTGTATAAAGGCTGTAACCCTTCTGTACATGCATGAACATACCCTTATTTGTTGTGTGTTGTCC
TCCTAAAAAAAAAAAAAAAAAAAAAAAA

taVP1

FIG. 10(a)

GTCCAGAGCCAGCCCCATGGCCAGCTCATGGTCCAGGTCCCGAATCCGCTGTCGACGAAGTCCA
ATTCCTCAAGGCAGAAGCAGCAAAAACCCTCGCCGGACGCAGCAGCGAGGCCGCCCTCCGGCGG
CGCCGCCACGCCGCAGCGCCCGGGGCAGGCGGCGGCTTCCGACAAGCAGCGGCAGCAGGGTGCG
AGGACGCCGGCGGCGGCGCCGGCGGCAGGAGACAAGAACCCGCGGTTCCTGCTGCAGAAGGTGC
TCAAGCAGAGCGACGTCGGAACCCTCGGCCGCATCGTGCTCCCCAAAGAAGCGGAGACTCACCT
GCCGGAGCTCAAGACGGGGGACGGCATCTCGATCCCCATTGAGGACATCGGCACATCTCAGGTG
TGGAGCATGCGATTTTGGCCCAACAACAAGAGCAGAATGTATCTTCTAGAGAACACTGGTGACT
TTGTTCGGTCGAATGAGCTGCAGGAGGGTGATTTCATCGTGCTTTACTCTGATGTCAAGTCGGG
CAAATATCTGATACGCGGCGTGAAGGTGAGAGCGCAACAGGATCTAGCCAAGCACAAGAATGCC
AGTCCAGAGAAAGGCGGGGCGTCCGACGTGAAGGCGGGCGGAGAAGACGGCGGCTGCAAGGAGA
AGCCCCCCCACGGCGTCCGGCGATCTCGCCAGGAGGCCGCCTCCATGAACCAGATGGCGGTGAG
CATCTGAAATGAGCAGGCTCGCCGTCCGATCCACCATTGAAGACTCAGTTAGCTAGCTCAAGTA
TACCCGTTGATGATGATCAAATCGATCTCTCGTTCTATGATCCGTGCTTCCGTGTACTGCTGTA
GCCCTAGTTAGGGATGATGATACTAAAGTAGCTATCGGTCAGATGTGACGCTGAAGAATGCATG
GTCCGTGCTGTTAAACCTGTAAAAGAAAAAAAAAAAAAAAGAAAAAGAAAAAAAAAA

Clone 2

FIG. 10(b)

TGGTCGCAGCATGCCGTCCAGGGCCAGCCCCATGGCCAGCTCATGGTCCAGGTTCCGAATCCGC
TGTCGACGAAGTCCAATTCCTCGAGGCAGAAGCAGCAAAAACCCTCGCCGGATGCAGCAGCGAG
GCCGCCCTCCGGCGGCGCCGCCACGCAGCAGCGCCCGGGGCAGGCGGCGGCTTCCGACAAGCAG
CGGCAGCAGGGTGCGAGGACGCCGGCGGCGGCGCCGGCGGCAGGAGACAAGAACCTGCGGTTCC
TGCTGCAGAAGGTGCTCAAGCAGAGCGACGTCGGAACCCTCGGCCGCATCGTGCTCCCCAAAAA
GGAAGCGGAGACTCACCTGCCGGAGCTCAAGACGGGGGACGGCATCTCGATCCCCATTGAGGAC
ATCGGCACATCTCAGGTGTGGAGCATGCGGTACCGATTTTGGCCCAACAACAAGAGCAGAATGT
ATCTTCTGGAGAACACTGGAGACTTTGTTCGGTCGAATGAGCTGCAGGAGGGTGATTTCATCGT
GCTTTACTCTGATGTCAAGTCGGGCAAATATCTGATACGCGGCGTGAAGGTAAGAGCGCAACAG
GATCTAGCCAAGCACAAGAATGGCAGTCCAGAGAAAGGTGGGGCGTCCGACGCGAAGGCGGGCG
CAGAAGACGGTGGTTGCAAAGAGAAGTCTCCGCACGGTGTCCGGCGATCTCGCCAGGAGGCCGC
CTCCATGAACCAGATGGCCGTGAGCATCTGAAATGAGCAGGCTCGCGCGGTCCGATCCCCCATT
GAAGACTACTTAGCTAGCTCAAGTATACCTGTTGATGATGATCAAATCGATCTCCCGTTCTATG
ATCCGTGCTTCCGTGTACTGCTGTAGCCCTAGTTAGGGATGGTGATACTAAAGTAGCTATCGGT
CAGATGTGACGCTGAAGAATGCATGGTCCGTGCTGTTAAACCTGTATAAAGGCTGTAACCCTTC
TGTAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA

Clone 3

FIG. 10(c)

GGCACGAGCCGCAGCGGATGGAGCCGGCGGCGACCAGGGAGGCCCGGAAGAAGAGGATGGCGAG
GCAGCGGCGCCTGTCGTGCCTGCAGCAGCAGCGGAGCCAGCAGCTGAATCTGAGCCAGATCCAA
ACCGGCGGCTTCCCTCAAGAGCCATCCCCCCGCGCGGCGCACTCGGCGCCGGTCACGCCGCCGT
CGTCTGGCTGGGGAGGCCTCTGGACGCAACAAGCCGTCCAGAGCCAGCCCCATGGCCAGCTCAT
GGTCCAGGTCCCGAATCCGCTGTCGACGAAGTCCAATTCCTCAAGGCAGAAGCAGCAAAAACCC
TCGCCGGACGCAGCAGCGAGGCCGCCCTCCGGCGGCGCCGCCACGCCGCAGCGCCCGGGGCAGG
CGGCGGCTTCCGACAAGCAGCGGCAGCAGGGTGCGAGGACGCCGGCGGCGGCGCCGGCGGCAGG
AGACAAGAACCCGCGGTTCCTGCTGCAGAAGGTGCTCAAGCAGAGCGACGTCGGAACCCTCGGC
CGCATCGTGCTCCCCAAAAAGGAAGCGGAGACTCACCTGCCGGAGCTCAAGACGGGGGACGGCA
TCTCGATCCCCATTGAGGACATCGGCACATCTCAGATTTTGGCCCAACAACAAGAGCAGAATGT
ATCTTCTAGAGAACACTGGTGACTTTGTTCGGTCGAATGAGCTGCAGGAGGGTGATTTCATCGT
GCTTTACTCTGATGTCAAGTCGGGCAAATATCTGATACGCGGCGTGAAGGTGAGAGCGCAACAG
GATCTAGCCAAGCACAAGAATGCCAGTCCAGAGAAAGGCGGGGCGTCCGACGTGAAGGCGGGCG
GAGAAGACGGCGGCTGCAAGGAGAAGCCCCCCCACGGCGTCCGGCGATCTCGCCAGGAGGCCGC
CTCCATGAACCAGATGGCGGTGAGCATCTGAAATGAGCAGGCTCGCCGTCCGATCCACCATTGA
AGACTCAGTTAGCTAGCTCAAGTATACCCGTTGATGATGATCAAATCGATCTCTCGTTCTATGA
TCCGTGCTTCCGTGTACTGCTGTAGCCCTAGTTAGGGATGATGATACTAAAGTAGCTATCGGTC
AGATGTGACGCTGAAGAATGCATGGTCCGTGCTGTTAAACCTGTATAAAGGCTGTAACCCTTCT
GTAAAAAAAAAAAAAAAAAAA

Clone 4

FIG. 10(d)

GGCACGAGGCGGCGCCTGTCGTGCCTGCAGCAGCAGCGGAGCCAGCAGCTGAATCTGAGCCAGA
TCCAAACCGGCGGCTTCCCTCAAGAGCCATCCCCCCGCGCGGCGCACTCGGCGCCGGTCACTCC
GCCGTCGTCTGGCTGGGGAGGCCTCTGGACGCAACAAGCCGTCCAGAGCCAGCCCCATGGCCAG
CTCATGGTCCAGGTCCCGAATCCGCTGTCGACGAAGTCCAATTCCTCAAGGCAGAAGCAGCAAA
AACCCTCGCCGGACGCAGCAGCGAGGCCGCCCTCCGGCGGCGCCGCCACGCCGCAGCGCCCGGG
GCAGGCGGCGGCTTCCGACAAGCAGCGGCAGCAGGGTGCGAGGACGCCGGCGGCGGCGCCGGCG
GCAGGAGACAAGAACCCGCGGTTCCTGCTGCAGAAGGTGCTCAAGCAGAGCGACGTCGGAACCC
TCGGCCGCATCGTGCTCCCCAAAGAAGCGGAGACTCACCTGCCGGAGCTCAAGACGGGGGACGG
CATCTCGATCCCCATTGAGGACATCGGCACATCTCAGATTTTGGCCCAACAACAAGAGCAGAAT
GTATCTTCTAGAGAACACTGGTGACTTTGTTCGGTCGAATGAGCTGCAGGAGGGTGATTTCATC
GTGCTTTACTCTGATGTCAAGTCGGGCAAATATCTGATACGCGGCGTGAAGGTGAGAGCGCAAC
AGGATCTAGCCAAGCACAAGAATGCCAGTCCAGAGAAAGGCGGGGCGTCCGACGTGAAGGCGGG
CGGAGAAGACGGCGGCTGCAAGGAGAAGCCCCCCCACGGCGTCCGGCGATCTCGCCAGGAGGCC
GCCTCCATGAACCAGATGGCGGTGAGCATCTGAAATGAGCAGGCTCGCCGTCCGATCCACCATT
GAAGACTCAGTTAGCTAGCTCAAGTATACCCGTTGATGATGATCAAATCGATCTCTCGTTCTAT
GATCCGTGCTTCCGTGTACTGCTGTAGCCCTAGTTAGGGATGATGATACTAAAGTAGCTATCGG
TCAGATGTGACGCTGAAGAATGCATGGTCCGTGCTGTTAAACCTGTATAAAGGCTGTAACCCTT
CTGTACATGCATGAACATACCCTTAAAAAAAAAAAAAAAAAAAAAA

Clone 5

FIG. 10(e)

CGCAGCGGATGGAACCGGCGGCGACCAGGGAGGCCCGGAAGAAGAGGATGGCGAGGCAGCGGCG
CCTGTCGTGCCTGCAGCAGCAGCGGAGCCAGCAGCTGAATCTGAGCCAGATCCAAAGCGGCGGC
TTCCCTCAAGAACCATCCCCCCGCGCGGCGCACTCGGCGCCGGTCACGCCGCCCTCTTCCGGCT
GGGGAGGCCTCTGGTCGCAGCATGCCGTCCAGGGCCAGCCCCATGGCCAGCTCATGGTCCAGGT
TCCGAATCCGCTGTCGACGAAGTCCAATTCCTCGAGGCAGAAGCAGCAAAAACCCTCGCCGGAT
GCAGCAGCGAGGCCGCCCTCCGGCGGCGCCGCCACGCAGCAGCGCCCGGGGCAGGCGGCGGCTT
CCGACAAGCAGCGGCAGCAGGGTGCGAGGACGCCGGCGGCGGCGCCGGCGGCAGGAGACAAGAA
CCTGCGGTTCCTGCTGCAGAAGGTGCTCAAGCAGAGCGACGTCGGAACCCTCGGCCGCATCGTG
CTCCCCAAAGAAGCGGAGACTCACCTGCCGGAGCTCAAGACGGGGGACGGCATCTCGATCCCCA
TTGAGGACATCGGCACATCTCAGGTGTGGAGCATGCGGTACCGATTTTGGCCCAACAACAAGAG
CAGAATGTATCTTCTGGAGAACACTGGAGACTTTGTTCGGTCGAATGAGCTGCAGGAGGGTGAT
TTCATCGTGCTTTACTCTGATGTCAAGTCGGGCAAATATCTGATACGCGGCGTGAAGGTAAGAG
CGCAACAGGATCTAGCCAAGCACAAGAATGGCAGTCCAGAGAAAGGTGGGGCGTCCGACGCGAA
GGCGGGCGCAGAAGACGGTGGTTGCAAAGAGAAGTCTCCGCACGGTGTCCGGCGATCTCGCCAG
GAGGCCGCCTCCATGAACCAGATGGCCGTGAGCATCTGAAATGAGCAGGCTCGCGCGGTCCGAT
CCCCCATTGAAGACTACTTAGCTAGCTCAAGTATACCTGTTGATGATGATCAAATCGATCTCCC
GTTCTATGATCCGTGCTTCCGTGTACTGCTGTAGCCCTAGTTAGGGATGGTGATACTAAAGTAG
CTATCGGTCAGATGTGACGCTGAAGAATGCATGGTCCGTGCTGTTAAACCTGTATAAAGGCTGT
AACCCTTCTGTTAAAAAAAAAAAAAAAAAAAAA

Clone 6

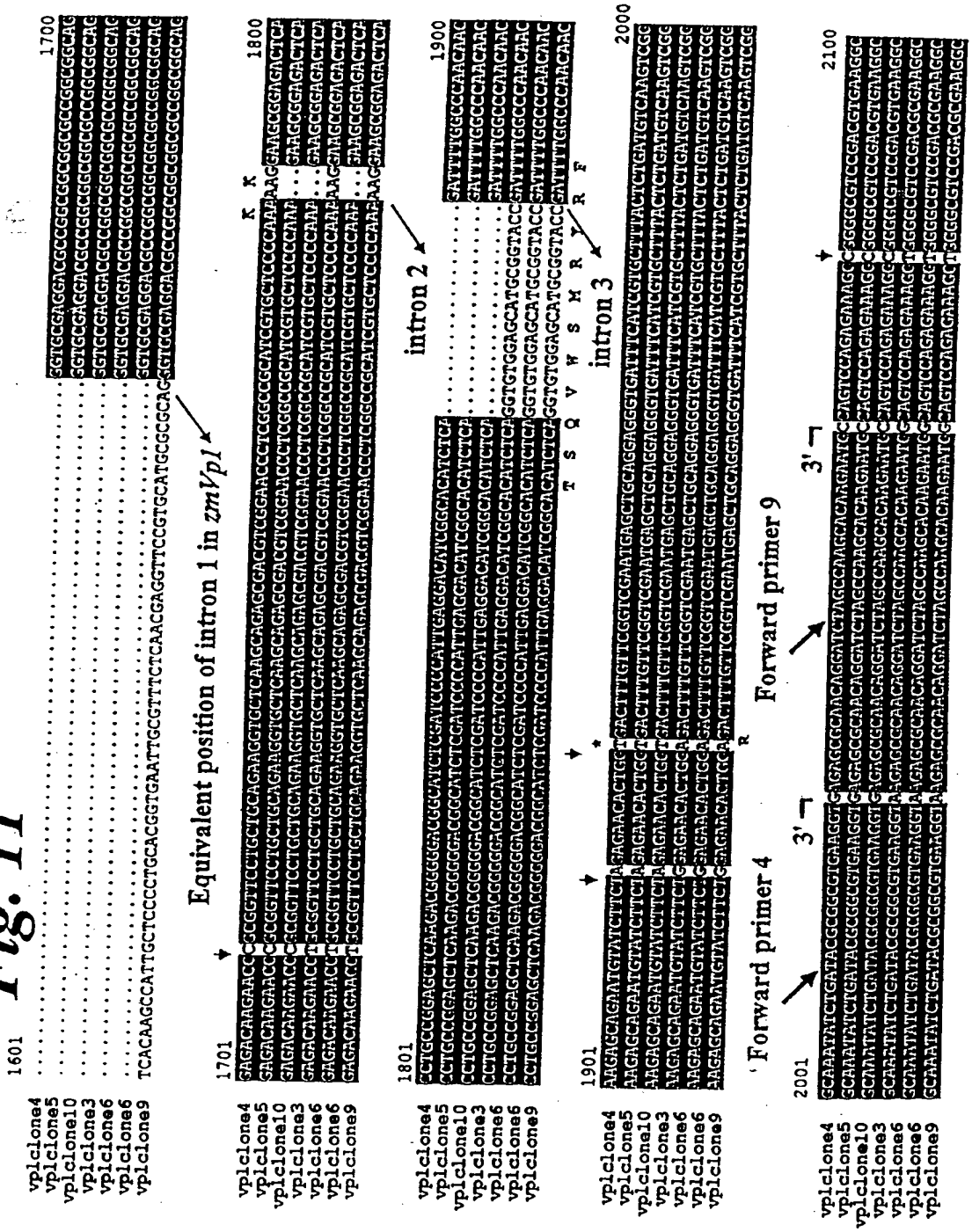
FIG. 10(F)

GGCACGAGCCACCATCGAGGCCGCGGCCGCGCGCCTCGGTGGGGGGCGCCAGGGCACCATGCAG
CTGCTCAAGCTCATCCTCACCTGGGTGCAGAACCACCACCTGCAGAAGAAGCGCCCCCGCGTCG
GCGCCATGGATCAGGAGGCGCCGCCGGCAGGAGGCCAGCTCCCCAGCCCCGGCGCAAACCCCGG
CTACGAATTCCCCGCGGAGACGGGTGCCGCCGCTAACACATCTTGGATGCCCTACCAGGCCTTC
TCGCCAACTGGATCCTACGGCGGCGAGGCGATCTACCCGTTCCAGCAGGGCTGCAGCACGAGCA
GCGTGGCCGTGAGCAGCCAGCCGTTCTCCCCGCCGGCGGCGCCCGACATGCACGCCGGGGCCTG
GCCGCTTCAGTACGCGGCGTTCGTCCCAGCTGGGGCCACATCCGCAGGCACTCAAACATACCCG
ATGCCGCCGCCGGGGGCCGTGCCGCAGCCGTTCGCGGCCCCCGGATTCGCCGGGCAGTTCCCGC
AGCGGATGGAACCGGCGGCGACCAGGGAGGCCCGGAAGAAGAGGATGGCGAGGCAGCGGCGCCT
GTCGTGCCTGCAGCAGCAGCGGAGCCAGCAGCTGAATCTGAGCCAGATCCAAAGCGGCGGCTTC
CCTCAAGAACCATCCCCCCCGCGCGGCGCACTCGGCGCCGGTCACGCCGCCCTCTTCCGGCTGGG
GAGGCCTCTGGTCGCAGCATGCCGTCCAGGGCCAGCCCCATGGCCAGCTCATGGTCCAGGTTCC
GAATCCGCTGTCGACGAAGTCCAATTCCTCGAGGCAGAAGCAGCAAAAACCCTCGCCGGATGCA
GCAGCGAGGCCGCCCTCCGGCGGCGCCGCCACGCAGCAGCGCCCGGGGCAGGCGGCGGCTTCCG
ACAAGCAGCGGCAGCAGGTGCATGCATGCACGAACACCTCTTGCCATCCATCCATCGATCGCCA
TCCCGCATAGAATCACAAGCCATTGCTCCCCAAATAAGTGTGCGTACATCGTAAGAGACGCACA
TCGCTGTCCAGCGATAGGATATCCCCGCATCGCCATCCCGCATAGAATCACAAGCCATTGCTCC
CCTGCACGGTGAATTGCGTTTCTCAACGAGGTTCCGTGCATGCGCGCAGGGTGCGAGGACGCCG
GCGGCGGCGCCGGCGGCAGGAGACAAGAACCTGCGGTTCCTGCTGCAGAAGGTGCTCAAGCAGA
GCGACGTCGGAACCCTCGGCCGCATCGTGCTCCCCAAAAAGGAAGCGGAGACTCACCTGCCGGA
GCTCAAGACGGGGGACGGCATCTCGATCCCCATTGAGGACATCGGCACATCTCAGGTGTGGAGC
ATGCGGTACCGATTTTGGCCCAACAACAAGAGCAGAATGTATCTTCTGGAGAACACTGGAGACT
TTGTTCGGTCGAATGAGCTGCAGGAGGGTGATTTCATCGTGCTTTACTCTGATGTCAAGTCGGG
CAAATATCTGATACGCGGCGTGAAGGTAAGAGCGCAACAGGATCTAGCCAAGCACAAGAATGGC
AGTCCAGAGAAAGGTGGGGCGTCCGACGCGAAGGCGGGCGCAGAAGACGGTGGTTGCAAAGAGA
AGTCTCCGCACGGTGTCCGGCGATCTCGCCAGGAGGCCGCCTCCATGAACCAGATGGCCGTGAG
CATCTGAAATGAGCAGGCTCGCGCGGTCCGATCCCCCATTGAAGACTACTTAGCTAGCTCAAGT
ATACCTGTTGATGATGATCAAATCGATCTCCCGTTCTATGATCCGTGCTTCCGTGTACTGCTGT
AGCCCTAGTTAGGGATGGTGATACTAAAGTAGCTATCGGTCAGATGTGACGCTGAAGAATGCAT
GGTCCGTGCTGTTAAACCTGTATAAAGGCTGTAACCCTTCTGTAAAAAAAAAAAAAAAAA

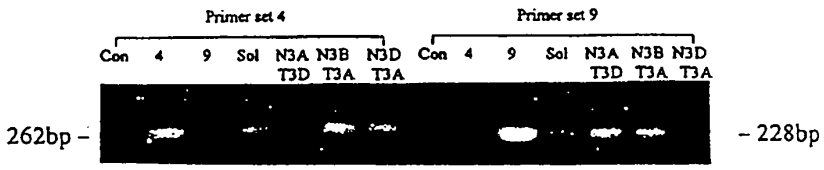
Clone 9

FIG. 10(g)

Fig. 11


GROUP I
GROUP II
Equivalent position of intron 1 in zmVp1
intron 2
intron 3
Forward primer 4
Forward primer 9
3'
3'
vp1clone4
vp1clone5
vp1clone10
vp1clone3
vp1clone6
vp1clone9

Assignment of *Vp1* cDNA clones to the 3A and 3D genomes of Chinese Spring



Summary:

	N3A T3D	N3B T3A	N3D T3A
Primer set 4	-	+	+
Primer set 9	+	+	-

FIG. 12

INTERNATIONAL SEARCH REPORT

Ir ational Application No
PCT/GB 98/02835

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12N15/29 C12N15/82 C12N5/10 C12Q1/68 C12C1/18
A01H5/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07K C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	"AC Q75687" EMBL DATABASE,4 August 1995, XP002093365 Heidelberg see the whole document ---	6,7
A	JOHNSON R. ET AL.: "Characterization of cDNA clones for differentially expressed genes in embryos of dormant and nondormant Avena fatua L. caryopses" PLANT MOLECULAR BIOLOGY, vol. 28, no. 1, April 1995, pages 113-122, XP002093366 cited in the application see the whole document --- -/--	1-75

[X] Further documents are listed in the continuation of box C. [ ] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
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"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
15 February 1999	25/02/1999

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Kania, T

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	LI B. AND FOLEY M.: "Cloning and characterization of differentially expressed genes in imbibed dormant and afterripened Avena fatua embryos" PLANT MOLECULAR BIOLOGY, vol. 29, no. 4, 4 November 1995, pages 823-831, XP002093367 cited in the application see the whole document	1-75
A	HATTORI T. ET AL.: "Sequence and functional analysis of te rice gene homologous to the maize Vp1" PLANT MOLECULAR BIOLOGY, vol. 24, no. 5, March 1994, pages 805-810, XP002063681 cited in the application see the whole document, esp. p.808 r. col.	1-75
A	BOBB A. ET AL.: "PvAlf, an embryo-specific acidic transcriptional activator enhances gene expression from phaseolin and phytohemagglutinin promoters" PLANT JOURNAL, vol. 8, no. 3, 1995, pages 331-343, XP002063683 cited in the application see the whole document	1-75
A	HOECKER U. ET AL.: "Integrated control of seed maturation and germination programs by activator and repressor functions of viviparous-1 of maize" GENES & DEVELOPMENT, vol. 9, no. 20, 15 October 1995, pages 2459-2469, XP002093368 cited in the application see the whole document	1-75
A	SORRELLS M. AND WILSON W.: "Direct classification and selection of superior alleles for crop improvement" CROP SCIENCE, vol. 37, no. 3, May 1997, pages 691-697, XP002093369 see the whole document, esp. p.693/94	1-75

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INTERNATIONAL SEARCH REPORT

In tional Application No
PCT/GB 98/02835

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	JONES H D ET AL: "Genotype and environment interact to control dormancy and differential expression of the VIVIPAROUS 1 homologue in embryo of Avena fatua." PLANT JOURNAL, (1997 OCT) 12 (4) 911-20. JOURNAL CODE: BRU. ISSN: 0960-7412., XP002093370 ENGLAND: United Kingdom see the whole document ---	1-75
P,X	MCKIBBIN, R. S. (1) ET AL: "Molecular analysis of the wheat viviparous 1 (VP1) homologue." JOURNAL OF EXPERIMENTAL BOTANY, (MAY, 1998) VOL. 49, NO. SUPPL., PP. 13. MEETING INFO.: THE SOCIETY FOR EXPERIMENTAL BIOLOGY ANNUAL MEETING LONDON ENGLAND, UK MARCH 22-27, 1998 SOCIETY FOR EXPERIMENTAL BIOLOGY. ISSN: 0022-0957., XP002093371 see abstract -----	1-75

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